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METHODS FOR EVALUATING OLIGONUCLEOTIDE PROBE SEQUENCES

Appendix

This patent application includes an appendix (the "Appendix"), which contains the source code for the software used in carrying out the examples in accordance with the present invention.

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BACKGROUND OF THE INVENTION

1. Field of the Invention.

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Significant morbidity and mortality are associated with infectious diseases and genetically inherited disorders. More rapid and accurate diagnostic methods are required for better monitoring and treatment of these conditions. Molecular methods using DNA probes, nucleic acid hybridization and *in vitro* amplification techniques are promising methods offering advantages to conventional methods used for patient diagnoses.

Nucleic acid hybridization has been employed for investigating the identity and establishing the presence of nucleic acids. Hybridization is based on

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complementary base pairing. When complementary single stranded nucleic acids are incubated together, the complementary base sequences pair to form doublestranded hybrid molecules. The ability of single stranded deoxyribonucleic acid (ssDNA) or ribonucleic acid (RNA) to form a hydrogen bonded structure with a complementary nucleic acid sequence has been employed as an analytical tool in molecular biology research. The availability of radioactive nucleoside triphosphates of high specific activity and the development of methods for their incorporation into DNA and RNA has made it possible to identify, isolate, and characterize various nucleic acid sequences of biological interest. Nucleic acid hybridization has great potential in diagnosing disease states associated with unique nucleic acid sequences. These unique nucleic acid sequences may result from genetic or environmental change in DNA by insertions, deletions, point mutations, or by acquiring foreign DNA or RNA by means of infection by bacteria, molds, fungi, and viruses. The application of nucleic acid hybridization as a diagnostic tool in clinical medicine is limited due to the cost and effort associated with the development of sufficiently sensitive and specific methods for detecting potentially low concentrations of disease-related DNA or RNA present in the complex mixture of nucleic acid sequences found in patient samples.

One method for detecting specific nucleic acid sequences generally involves immobilization of the target nucleic acid on a solid support such as nitrocellulose paper, cellulose paper, diazotized paper, or a nylon membrane. After the target nucleic acid is fixed on the support, the support is contacted with a suitably labeled probe nucleic acid for about two to forty-eight hours. After the above time period, the solid support is washed several times at a controlled temperature to remove unhybridized probe. The support is then dried and the hybridized material is detected by autoradiography or by spectrometric methods. When very low concentrations must be detected, the above method is slow and labor intensive, and nonisotopic labels that are less readily detected than radiolabels are frequently not suitable.

A method for the enzymatic amplification of specific segments of DNA known as the polymerase chain reaction (PCR) method has been described. This *in vitro* amplification procedure is based on repeated cycles of denaturation, oligonucleotide primer annealing, and primer extension by thermophilic

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polymerase, resulting in the exponential increase in copies of the region flanked by the primers. The PCR primers, which anneal to opposite strands of the DNA, are positioned so that the polymerase catalyzed extension product of one primer can serve as a template strand for the other, leading to the accumulation of a discrete fragment whose length is defined by the distance between the 5' ends of the oligonucleotide primers.

Other methods for amplifying nucleic acids have also been developed. These methods include single primer amplification, ligase chain reaction (LCR), transcription-mediated amplification methods including 3SR and NASBA, and the Q-beta-replicase method. Regardless of the amplification used, the amplified product must be detected.

One method for detecting nucleic acids is to employ nucleic acid probes that have sequences complementary to sequences in the target nucleic acid. A nucleic acid probe may be, or may be capable of being, labeled with a reporter group or may be, or may be capable of becoming, bound to a support. Detection of signal depends upon the nature of the label or reporter group. Usually, the probe is comprised of natural nucleotides such as ribonucleotides and deoxyribonucleotides and their derivatives although unnatural nucleotide mimetics such as peptide nucleic acids and oligomeric nucleoside phosphonates are also used. Commonly, binding of the probes to the target is detected by means of a label incorporated into the probe. Alternatively, the probe may be unlabeled and the target nucleic acid labeled. Binding can be detected by separating the bound probe or target from the free probe or target and detecting the label. In one approach, a sandwich is formed comprised of one probe, which may be labeled, the target and a probe that is or can become bound to a surface. Alternatively, binding can be detected by a change in the signal-producing properties of the label upon binding, such as a change in the emission efficiency of a fluorescent or chemiluminescent label. This permits detection to be carried out without a separation step. Finally, binding can be detected by labeling the target, allowing the target to hybridize to a surface-bound probe, washing away the unbound target and detecting the labeled target that remains.

Direct detection of labeled target hybridized to surface-bound probes is particularly advantageous if the surface contains a mosaic of different probes that

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are individually localized to discrete, known areas of the surface. Such ordered arrays containing a large number of oligonucleotide probes have been developed as tools for high throughput analyses of genotype and gene expression. Oligonucleotides synthesized on a solid support recognize uniquely complementary nucleic acids by hybridization, and arrays can be designed to define specific target sequences, analyze gene expression patterns or identify specific allelic variations. One difficulty in the design of oligonucleotide arrays is that oligonucleotides targeted to different regions of the same gene can show large differences in hybridization efficiency, presumably due, at least in part, to the interplay between the secondary structures of the oligonucleotides and their targets and the stability of the final probe/target hybridization product. A method for predicting which oligonucleotides will show detectable hybridization would substantially decrease the number of iterations required for optimal array design and would be particularly useful when the total number of oligonucleotide probes on the array is limited. A method to predict oligonucleotide hybridization efficiency would also streamline the empirical approaches currently used to select potential antisense therapeutics, which are designed to modulate gene expression in vivo by hybridizing to specific messenger RNA (mRNA) molecules and inhibiting their translation into proteins.

While it is well known that the structure of the target nucleic acid affects the affinity of oligonucleotide hybridization, current methods for predicting target structures from the primary sequence fail to predict target regions accessible for oligonucleotide binding. Consequently, selection of oligonucleotides for antisense reagents or oligonucleotide probe arrays has been largely empirical. As most of the target sequence is sequestered by intramolecular base pairing and not accessible for oligonucleotide binding, the process of identifying good oligonucleotides has required large numbers of low efficiency experiments.

The design and implementation of algorithms that effectively predict the ability of oligonucleotides to rapidly and avidly bind to complementary nucleotide sequences has been an important problem in molecular biology since the invention of facile methods for chemical DNA synthesis. The subsequent inventions of the polymerase chain reaction (PCR), antisense inhibition of gene expression and oligonucleotide array methods for performing massively parallel

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hybridization experiments have made the need for effective predictive algorithms even more critical.

Previous attempts to solve the nucleic acid probe design problem include PCR primer design software applications (e.g., OLIGO®), neural networks, PCR primer design applications that search for sequences that possess minimal ability to cross-hybridize with other targets present in a sample (e.g., HYBsimulator™), and approaches that attempt to predict the efficiency of antisense sequence suppression of mRNA translation from a combination of predicted nucleic acid duplex melting temperature and predicted target strand structure. The methods that predict effective oligonucleotide primers for performing PCR from DNA templates work well for that application where relatively stringent conditions are employed. This is because PCR experimental design greatly simplifies the prediction problem: hybridization is performed at high temperature, at relatively low ionic strength and in the presence of a large molar excess of oligonucleotide. Under these conditions, the oligonucleotide and target secondary structures are relatively unimportant.

Unfortunately, these conditions do not apply to oligonucleotide arrays, which are usually hybridized under relatively non-denaturing conditions, or to antisense suppression of gene expression, which takes place *in vivo*.

Oligonucleotide arrays can contain hundreds of thousands of different sequences and conditions are chosen to allow the oligonucleotide with the lowest melting temperature to hybridize efficiently. These "lowest common denominator" conditions are usually relatively non-denaturing and secondary structure constraints become significant. Accordingly, the above applications require new predictive methods that are capable of estimating the effects of oligonucleotide and target structure on hybridization efficiency. For these reasons, current algorithms for designing PCR primer oligonucleotides fail badly when applied to the problems of oligonucleotide array or antisense oligonucleotide design.

To date, the most effective approach for identifying oligonucleotides with good hybridization efficiency has been an empirical one. Such an approach involves the synthesis of large numbers of oligonucleotide probes for a given target nucleotide sequence. Arrays are formed that include the above oligonucleotide probes. Hybridization experiments are carried out to determine

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which of the oligonucleotide probes exhibit good hybridization efficiencies. Examples of such an approach are found in D. Lockhart, et al., Nature Biotech., infra, L. Wodicka, et al., Nature Biotechnology, infra., and N. Milner et al. Nature Biotech, infra. One major drawback to this approach is the vast number of oligonucleotides that must be synthesized in order to achieve a satisfactory result. Typically, about 2%-5% of the test probes synthesized yield acceptable signal levels.

The use of neural networks for oligonucleotide design has also been investigated. Neural networks are easily taught with real data; they therefore afford a general approach to many problems. However, their performance is limited by the "senses" that they are given. An analogy works best here: the human brain is an astoundingly capable neural network, but a blind person cannot be taught to reliably distinguish colors by smell. In addition, a large amount of data is required to adequately teach a neural network to perform its job well. A comprehensive database for either oligonucleotide array design or antisense suppression of gene expression has not been made available. For these reasons, the performance reported to-date of neural network solutions against the probe design problem is mediocre.

Finally, approaches that have attempted to use target nucleic acid folding calculations to predict experimental results inferred to depend upon hybridization efficiency (e.g. antisense suppression of mRNA translation) have so far only demonstrated that the predictions of current nucleic acid folding calculations correlate poorly with observed behavior. The probable reason for this is that the structures predicted by such programs for long sequences are poor predictors of chemical reality; the results of experiments that attempt to confirm the predictions of such calculations support this assessment. Recent improvements to this approach which use predicted RNA structure topology as a predictor of relative RNA/RNA association kinetics have been more successful at forecasting the results of antisense experiments. However, these methods are not computationally efficient, and have so far only been shown to work for targets less than 100 bases long. Such methods are therefore not yet capable of predicting the behavior of full-length mRNA targets, which are typically between 1,000 and 2,000 bases in length.

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2. Description of the Related Art.

U.S. Patent No. 5,512,438 (Ecker) discloses the inhibition of RNA expression by forming a pseudo-half knot RNA at the target's RNA secondary structure using antisense oligonucleotides.

Cook, et al., in U.S. Patent No. 5,670,633 discuss sugar-modified oligonucleotides that detect and modulate gene expression.

Antisense oligonucleotide inhibition of the RAS gene is disclosed in U.S. Patent No. 5,582,986 (Monia, *et al.*).

U.S. Patent No. 5,593,834 (Lane, *et al.*) discusses a method of preparing DNA sequences with known ligand binding characteristics.

Mitsuhashi, et al., in U.S. Patent No. 5,556,749 discusses a computerized method for designing optimal DNA probes and an oligonucleotide probe design station.

U.S. Patent No. 5,081,584 (Omichinski, *et al.*) discloses a computer-assisted design of anti-peptides based on the amino acid sequence of a target peptide.

A PCR primer design application that searches for sequences that possess minimal ability to cross-hybridize with other targets present in a sample is available as HYBsimulator™, version 2.0, AGCT, Inc., 2102 Business Center Drive, Suite 170, Irvine, CA 92715 (714) 833-9983.

A PCR primer design software application is available as OLIGO®, version 5.0, National Biosciences, Inc., 3650 Annapolis Lane North, #140, Plymouth, MN 55447 (800) 747-4362.

- D. J. Lockhart, et al., <u>Nature Biotech</u>. <u>14</u>:1675-1684 (1996) describe a neural network approach to the selection of efficient surface-bound oligonucleotide probes.
- M. Mitsuhashi, *et al.*, <u>Nature</u>, <u>367</u>:759-761 (1994) disclose a method for designing specific oligonucleotide probes and primers by modeling the potential cross-hybridization of candidate probes to non-target sequences known to be present in samples.
- R. A. Stull, *et al.*, <u>Nuc. Acids Res.</u>, <u>20</u>:3501-3508 (1992) describe a method of predicting the efficacy of antisense oligonucleotides, using predicted target

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secondary structure and predicted oligonucleotide/target binding free energy as input parameters.

- N. Milner, et al., Nature Biotechnology, 15:537-541 (1997) compare observed patterns of probe hybridization to those expected from the predicted secondary structure of the nucleic acid target.
- L. Wodicka, *et al.*, <u>Nature Biotechnology</u>, <u>15</u>:1359-1367 (1997) describe simple rules for avoiding inefficient and non-specific probes during design and synthesis of oligonucleotides arrays.
- J. SantaLucia Jr., et al., <u>Biochemistry</u>, <u>35</u>:3555 (1996) disclose parameters and methods for the calculation of thermodynamic properties of DNA/DNA homoduplexes.
 - N. Sugimoto, *et al.*, <u>Biochemistry</u>, <u>34</u>:11211 (1995) disclose parameters and methods for the calculation of thermodynamic properties of DNA/RNA heteroduplexes.
 - J.A. Jaeger, et al., <u>Proc. Natl. Acad. Sci. USA</u>, <u>86</u>:7706 (1989) disclose methods for estimation of the free energy of the most stable intramolecular structure of a single-stranded polynucleotide, by means of a dynamic programming algorithm.
 - S. F. Altschul, et al., <u>Nature Genetics</u>, <u>6</u>:119-129 (1994) disclose methods for calculating the complexity and information content of amino acid and nucleic acid sequences.
 - T. A. Weber and E. Helfand, <u>J. Chem. Phys.</u>, <u>71</u>, 4760 (1979) describe approaches for the modeling of polymer structures by molecular dynamics simulations.
 - V. Patzel and G. Sczakiel, <u>Nature Biotech.</u>, <u>16</u>, 64-68 (1998) disclose methods for estimating rate constants for association of antisense RNA molecules with mRNA targets by examination of predicted antisense RNA secondary structures.
- Light-generated oligonucleotide arrays for rapid DNA sequence analysis is described by A. C. Pease, *et al.*, <u>Proc. Nat. Acad. Sci. USA</u> (1994) <u>91</u>:5022-5026.

Mitsuhashi discusses basic requirements for designing optimal oligonucleotide probe sequences in <u>J. Clinical Laboratory Analysis</u> (1996) <u>10</u>:277-284.

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Rychlik, *et al.*, discloses a computer program for choosing optimal oligonucleotides for filter hybridization, sequencing and in vitro amplification of DNA in <u>Nucleic Acids Research</u> (1989) <u>17</u>(21):8543-8551.

A strategy for designing specific antisense oligonucleotide sequences is described by Mitsuhashi in <u>J. Gastroenterol</u>. (1997) 32:282-287.

Mitsuhashi discusses basic requirements for designing optimal PCR primers in J. Clinical Laboratory Analysis (1996) 10:285-293.

Hyndman, *et al.*, disclose software to determine optimal oligonucleotide sequences based on hybridization simulation data in <u>BioTechniques</u> (1996) 20(6):1090-1094.

Eberhardt discloses a shell program for the design of PCR primers using genetics computer group (GCG) software (7.1) on VAX/VMS™ systems in BioTechniques (1992) 13(6):914-917.

Chen, et al., disclose a computer program for calculating the melting temperature of degenerate oligonucleotides used in PCR or hybridization in BioTechniques (1997) 22(6):1158-1160.

Partial thermodynamic parameters for prediction stability and washing behavior of DNA duplexes immobilized on gel matrix is described by Kunitsyn, *et al.*, in J. Biomolecular Structure & Dynamics, ISSN 0739-1102 (1996) 14(1):239-244.

SUMMARY OF THE INVENTION

One embodiment of the present invention is a method for predicting the potential of an oligonucleotide to hybridize to a target nucleotide sequence. A predetermined set of unique oligonucleotide sequences is identified. The unique oligonucleotide sequences are chosen to sample the entire length of a nucleotide sequence that is hybridizable with the target nucleotide sequence. At least one parameter that is predictive of the ability of each of the oligonucleotides specified by the set of sequences to hybridize to the target nucleotide sequence is determined and evaluated for each of the above oligonucleotide sequences. A subset of oligonucleotide sequences within the predetermined set of unique oligonucleotide sequences is identified based on the examination of the parameter values. Finally, oligonucleotide sequences in the subset are identified that are

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clustered along one or more regions of the nucleotide sequence that is hybridizable to the target nucleotide sequence. The oligonucleotide probes corresponding to the identified sequences find use in polynucleotide assays particularly where the assays involve oligonucleotide arrays. For a discussion of oligonucleotide arrays, see, e.g., U.S. Patent No. 5,700,637 (E. Southern) and U.S. Patent No. 5,667,667 (E. Southern), the relevant disclosures of which are incorporated herein by reference.

Another embodiment of the present invention is a method for predicting the potential of an oligonucleotide to hybridize to a complementary target nucleotide sequence. A set of overlapping oligonucleotide sequences is identified based on a nucleotide sequence that is complementary to the target nucleotide sequence. At least two parameters that are independently predictive of the ability of each of the oligonucleotides specified by the oligonucleotide sequences to hybridize to the target nucleotide sequence are determined and evaluated for each of the oligonucleotide sequences. Independence is assured by requiring that the parameters be poorly correlated with respect to one another. A subset of oligonucleotide sequences within the set of oligonucleotide sequences is identified based on the examination of the parameter values. Finally, oligonucleotide sequences in the subset are identified that are clustered along one or more regions of the nucleotide sequence that is complementary to the target nucleotide sequence.

Another embodiment of the present invention is a method for predicting the potential of an oligonucleotide to hybridize to a complementary target nucleotide sequence. A set of overlapping oligonucleotide sequences is obtained based on a nucleotide sequence of length L, complementary to the target nucleotide sequence. The oligonucleotide sequences of the set of overlapping oligonucleotide sequences are of identical length N and spaced one nucleotide apart. The set comprises L-N+1 oligonucleotide sequences. Parameters are determined for each of the oligonucleotide sequences of the set of overlapping oligonucleotide sequences. One parameter is the predicted melting temperature of the duplex of each of the oligonucleotides specified by the oligonucleotide sequences and the target nucleotide sequence, corrected for salt concentration. The other parameter is the predicted free energy of the most stable intramolecular

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structure of each of the oligonucleotides specified by the oligonucleotide sequences at the temperature of hybridization of the oligonucleotide with the target nucleotide sequence. A subset of oligonucleotide sequences within the set of oligonucleotide sequences is selected based on an examination of the parameter values by establishing cut-off values for each of the parameters. Oligonucleotide sequences in the subset that are clustered along one or more regions of the complementary nucleotide sequence are ranked based on the sizes of the clusters of oligonucleotide sequences. Finally, a subset of the clustered oligonucleotide sequences is selected that statistically samples the clusters of oligonucleotide sequences. The selected sampled subset is used to specify the

synthesis of oligonucleotides for experimental evaluation.

Another aspect of the present invention is a computer based method for predicting the potential of an oligonucleotide to hybridize to a target nucleotide sequence. A predetermined number of unique oligonucleotides within a nucleotide sequence that is hybridizable with the target nucleotide sequence is identified under computer control. The oligonucleotides are chosen to sample the entire length of the nucleotide sequence. A value is determined and evaluated under computer control for each of the oligonucleotides for at least one parameter that is independently predictive of the ability of each of the oligonucleotides to hybridize to the target nucleotide sequence. The parameter values are stored. A subset of oligonucleotides within the predetermined number of unique oligonucleotides is identified by examination of the stored parameter values under computer control. Then, oligonucleotides in the subset that are clustered along a region of the nucleotide sequence that is hybridizable to the target nucleotide sequence are identified under computer control.

Another aspect of the present invention is a computer system for conducting a method for predicting the potential of an oligonucleotide to hybridize to a target nucleotide sequence. The system comprises (a) input means for introducing a target nucleotide sequence into the computer system, (b) means for determining a number of unique oligonucleotide sequences that are within a nucleotide sequence that is hybridizable with the target nucleotide sequence where the oligonucleotide sequences are chosen to sample the entire length of the nucleotide sequence, (c) memory means for storing the oligonucleotide





sequences, (d) means for controlling the computer system to carry out for each of the oligonucleotide sequences a determination and evaluation of a value for at least one parameter that is independently predictive of the ability of each of the oligonucleotide sequences to hybridize to the target nucleotide sequence, (e) means for storing the parameter values, (f) means for controlling the computer to carry out an identification from the stored parameter values a subset of oligonucleotide sequences within the number of unique oligonucleotide sequences based on the examination of the parameter, (g) means for storing the subset of oligonucleotides, (h) means for controlling the computer to carry out an identification of oligonucleotide sequences in the subset that are clustered along a region of the nucleotide sequence that is hybridizable to the target nucleotide sequence, (i) means for storing the oligonucleotide sequences in the subset, and (j) means for outputting data relating to the oligonucleotide sequences in the subset.

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BRIEF DESCRIPTION OF THE DRAWINGS

Fig.1 is a general flow chart depicting the method of the present invention.

Fig. 2 is a flow chart depicting a preferred embodiment of a method in accordance with the present invention.

Fig. 3 is a contour plot of normalized hybridization intensity from multiple experiments, as a function of the free energy of the most stable probe intramolecular structure (ΔG_{MFOLD}) and the difference between the predicted RNA/DNA heteroduplex melting temperature (T_m) and the temperature of hybridization (T_{hyb}).

Fig. 4 shows the observed hybridization patterns for oligonucleotides selected using a method in accordance with the present invention and additional oligonucleotides to a portion of the rabbit β -globin gene (radiolabeled antisense RNA target).

Fig. 5 shows the observed hybridization patterns for oligonucleotides selected using a method in accordance with the present invention and additional oligonucleotides to the HIV PRT gene (fluorescein-labeled sense RNA target).





Fig. 6 shows the observed hybridization patterns for oligonucleotides selected using a method in accordance with the present invention and additional oligonucleotides to the G3PDH gene (fluorescein-labeled antisense RNA target).

Fig. 7 shows the observed hybridization patterns for oligonucleotides selected using a method in accordance with the present invention and additional oligonucleotides to the p53 gene (fluorescein-labeled antisense RNA target).

Fig. 8 shows the observed hybridization patterns for oligonucleotides selected using a method in accordance with the present invention and additional oligonucleotides to the HIV PRTs gene (using data from the GeneChip™ data).

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DEFINITIONS

Before proceeding further with a description of the specific embodiments of the present invention, a number of terms will be defined.

Nucleic Acids:

Polynucleotide -- a compound or composition that is a polymeric nucleotide or nucleic acid polymer. The polynucleotide may be a natural compound or a synthetic compound. In the context of an assay, the polynucleotide is often referred to as a polynucleotide analyte. The polynucleotide can have from about 20 to 5,000,000 or more nucleotides. The larger polynucleotides are generally found in the natural state. In an isolated state the polynucleotide can have about 30 to 50,000 or more nucleotides, usually about 100 to 20,000 nucleotides, more frequently 500 to 10,000 nucleotides. It is thus obvious that isolation of a polynucleotide from the natural state often results in fragmentation. The polynucleotides include nucleic acids, and fragments thereof, from any source in purified or unpurified form including DNA (dsDNA and ssDNA) and RNA, including tRNA, mRNA, rRNA, mitochondrial DNA and RNA, chloroplast DNA and RNA, DNA/RNA hybrids, or mixtures thereof, genes, chromosomes, plasmids, the genomes of biological material such as microorganisms, e.g., bacteria, yeasts, viruses, viroids, molds, fungi, plants, animals, humans, and the like. The polynucleotide can be only a minor fraction of a complex mixture such as a biological sample. Also included are genes, such as hemoglobin gene for sicklecell anemia, cystic fibrosis gene, oncogenes, cDNA, and the like.

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The polynucleotide can be obtained from various biological materials by procedures well known in the art. The polynucleotide, where appropriate, may be cleaved to obtain a fragment that contains a target nucleotide sequence, for example, by shearing or by treatment with a restriction endonuclease or other site specific chemical cleavage method.

For purposes of this invention, the polynucleotide, or a cleaved fragment obtained from the polynucleotide, will usually be at least partially denatured or single stranded or treated to render it denatured or single stranded. Such treatments are well known in the art and include, for instance, heat or alkali treatment, or enzymatic digestion of one strand. For example, dsDNA can be heated at 90-100° C. for a period of about 1 to 10 minutes to produce denatured material.

Target nucleotide sequence -- a sequence of nucleotides to be identified, usually existing within a portion or all of a polynucleotide, usually a polynucleotide analyte. The identity of the target nucleotide sequence generally is known to an extent sufficient to allow preparation of various sequences hybridizable with the target nucleotide sequence and of oligonucleotides, such as probes and primers, and other molecules necessary for conducting methods in accordance with the present invention, an amplification of the target polynucleotide, and so forth.

The target sequence usually contains from about 30 to 5,000 or more nucleotides, preferably 50 to 1,000 nucleotides. The target nucleotide sequence is generally a fraction of a larger molecule or it may be substantially the entire molecule such as a polynucleotide as described above. The minimum number of nucleotides in the target nucleotide sequence is selected to assure that the presence of a target polynucleotide in a sample is a specific indicator of the presence of polynucleotide in a sample. The maximum number of nucleotides in the target nucleotide sequence is normally governed by several factors: the length of the polynucleotide from which it is derived, the tendency of such polynucleotide to be broken by shearing or other processes during isolation, the efficiency of any procedures required to prepare the sample for analysis (e.g. transcription of a DNA template into RNA) and the efficiency of detection and/or amplification of the target nucleotide sequence, where appropriate.

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Oligonucleotide -- a polynucleotide, usually single stranded, usually a synthetic polynucleotide but may be a naturally occurring polynucleotide. The oligonucleotide(s) are usually comprised of a sequence of at least 5 nucleotides, preferably, 10 to 100 nucleotides, more preferably, 20 to 50 nucleotides, and usually 10 to 30 nucleotides, more preferably, 20 to 30 nucleotides, and desirably about 25 nucleotides in length.

Various techniques can be employed for preparing an oligonucleotide. Such oligonucleotides can be obtained by biological synthesis or by chemical synthesis. For short sequences (up to about 100 nucleotides), chemical synthesis will frequently be more economical as compared to the biological synthesis. In addition to economy, chemical synthesis provides a convenient way of incorporating low molecular weight compounds and/or modified bases during specific synthesis steps. Furthermore, chemical synthesis is very flexible in the choice of length and region of the target polynucleotide binding sequence. The oligonucleotide can be synthesized by standard methods such as those used in commercial automated nucleic acid synthesizers. Chemical synthesis of DNA on a suitably modified glass or resin can result in DNA covalently attached to the surface. This may offer advantages in washing and sample handling. For longer sequences standard replication methods employed in molecular biology can be used such as the use of M13 for single stranded DNA as described by J. Messing (1983) Methods Enzymol, 101:20-78.

Other methods of oligonucleotide synthesis include phosphotriester and phosphodiester methods (Narang, et al. (1979) Meth. Enzymol 68:90) and synthesis on a support (Beaucage, et al. (1981) Tetrahedron Letters

22:1859-1862) as well as phosphoramidite techniques (Caruthers, M. H., et al., "Methods in Enzymology," Vol. 154, pp. 287-314 (1988)) and others described in "Synthesis and Applications of DNA and RNA," S.A. Narang, editor, Academic Press, New York, 1987, and the references contained therein. The chemical synthesis via a photolithographic method of spatially addressable arrays of oligonucleotides bound to glass surfaces is described by A. C. Pease, et al., Proc. Nat. Acad. Sci. USA (1994) 91:5022-5026.

Oligonucleotide probe -- an oligonucleotide employed to bind to a portion of a polynucleotide such as another oligonucleotide or a target nucleotide sequence.

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The design and preparation of the oligonucleotide probes are generally dependent upon the sensitivity and specificity required, the sequence of the target polynucleotide and, in certain cases, the biological significance of certain portions of the target polynucleotide sequence.

Oligonucleotide primer(s) — an oligonucleotide that is usually employed in a chain extension on a polynucleotide template such as in, for example, an amplification of a nucleic acid. The oligonucleotide primer is usually a synthetic nucleotide that is single stranded, containing a sequence at its 3'-end that is capable of hybridizing with a defined sequence of the target polynucleotide. Normally, an oligonucleotide primer has at least 80%, preferably 90%, more preferably 95%, most preferably 100%, complementarity to a defined sequence or primer binding site. The number of nucleotides in the hybridizable sequence of an oligonucleotide primer should be such that stringency conditions used to hybridize the oligonucleotide primer will prevent excessive random non-specific hybridization. Usually, the number of nucleotides in the oligonucleotide primer will be at least as great as the defined sequence of the target polynucleotide, namely, at least ten nucleotides, preferably at least 15 nucleotides, and generally from about 10 to 200, preferably 20 to 50, nucleotides.

In general, in primer extension, amplification primers hybridize to, and are extended along (chain extended), at least the target nucleotide sequence within the target polynucleotide and, thus, the target sequence acts as a template. The extended primers are chain "extension products." The target sequence usually lies between two defined sequences but need not. In general, the primers hybridize with the defined sequences or with at least a portion of such target polynucleotide, usually at least a ten-nucleotide segment at the 3'-end thereof and preferably at least 15, frequently a 20 to 50 nucleotide segment thereof.

Nucleoside triphosphates -- nucleosides having a 5'-triphosphate substituent. The nucleosides are pentose sugar derivatives of nitrogenous bases of either purine or pyrimidine derivation, covalently bonded to the 1'-carbon of the pentose sugar, which is usually a deoxyribose or a ribose. The purine bases include adenine (A), guanine (G), inosine (I), and derivatives and analogs thereof. The pyrimidine bases include cytosine (C), thymine (T), uracil (U), and derivatives and analogs thereof. Nucleoside triphosphates include deoxyribonucleoside

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triphosphates such as the four common deoxyribonucleoside triphosphates dATP, dCTP, dGTP and dTTP and ribonucleoside triphosphates such as the four common triphosphates rATP, rCTP, rGTP and rUTP.

The term "nucleoside triphosphates" also includes derivatives and analogs thereof, which are exemplified by those derivatives that are recognized and polymerized in a similar manner to the underivatized nucleoside triphosphates.

Nucleotide -- a base-sugar-phosphate combination that is the monomeric unit of nucleic acid polymers, i.e., DNA and RNA. The term "nucleotide" as used herein includes modified nucleotides as defined below.

DNA -- deoxyribonucleic acid.

RNA -- ribonucleic acid.

Modified nucleotide -- a unit in a nucleic acid polymer that contains a modified base, sugar or phosphate group. The modified nucleotide can be produced by a chemical modification of the nucleotide either as part of the nucleic acid polymer or prior to the incorporation of the modified nucleotide into the nucleic acid polymer. For example, the methods mentioned above for the synthesis of an oligonucleotide may be employed. In another approach a modified nucleotide can be produced by incorporating a modified nucleoside triphosphate into the polymer chain during an amplification reaction. Examples of modified nucleotides, by way of illustration and not limitation, include dideoxynucleotides, derivatives or analogs that are biotinylated, amine modified, alkylated, fluorophore-labeled, and the like and also include phosphorothioate, phosphite, ring atom modified derivatives, and so forth.

Nucleoside -- is a base-sugar combination or a nucleotide lacking a phosphate moiety.

Nucleotide polymerase -- a catalyst, usually an enzyme, for forming an extension of a polynucleotide along a DNA or RNA template where the extension is complementary thereto. The nucleotide polymerase is a template dependent polynucleotide polymerase and utilizes nucleoside triphosphates as building blocks for extending the 3'-end of a polynucleotide to provide a sequence complementary with the polynucleotide template. Usually, the catalysts are enzymes, such as DNA polymerases, for example, prokaryotic DNA polymerase (I, II, or III), T4 DNA polymerase, T7 DNA polymerase, Klenow fragment, reverse

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transcriptase, Vent DNA polymerase, \underline{Pfu} DNA polymerase, \underline{Taq} DNA polymerase, and the like, or RNA polymerases, such as T3 and T7 RNA polymerases. Polymerase enzymes may be derived from any source such as cells, bacteria such as \underline{E} . \underline{coli} , plants, animals, virus, thermophilic bacteria, and so forth.

Amplification of nucleic acids or polynucleotides -- any method that results in the formation of one or more copies of a nucleic acid or polynucleotide molecule (exponential amplification) or in the formation of one or more copies of only the complement of a nucleic acid or polynucleotide molecule (linear amplification).

Hybridization (hybridizing) and binding -- in the context of nucleotide sequences these terms are used interchangeably herein. The ability of two nucleotide sequences to hybridize with each other is based on the degree of complementarity of the two nucleotide sequences, which in turn is based on the fraction of matched complementary nucleotide pairs. The more nucleotides in a given sequence that are complementary to another sequence, the more stringent the conditions can be for hybridization and the more specific will be the binding of the two sequences. Increased stringency is achieved by elevating the temperature, increasing the ratio of co-solvents, lowering the salt concentration, and the like.

Hybridization efficiency -- the productivity of a hybridization reaction, measured as either the absolute or relative yield of oligonucleotide probe/polynucleotide target duplex formed under a given set of conditions in a given amount of time.

Homologous or substantially identical polynucleotides -- In general, two polynucleotide sequences that are identical or can each hybridize to the same polynucleotide sequence are homologous. The two sequences are homologous or substantially identical where the sequences each have at least 90%, preferably 100%, of the same or analogous base sequence where thymine (T) and uracil (U) are considered the same. Thus, the ribonucleotides A, U, C and G are taken as analogous to the deoxynucleotides dA, dT, dC, and dG, respectively. Homologous sequences can both be DNA or one can be DNA and the other RNA.

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Complementary -- Two sequences are complementary when the sequence of one can bind to the sequence of the other in an anti-parallel sense wherein the 3'-end of each sequence binds to the 5'-end of the other sequence and each A, T(U), G, and C of one sequence is then aligned with a T(U), A, C, and G, respectively, of the other sequence. RNA sequences can also include complementary G/U or U/G basepairs.

Member of a specific binding pair ("sbp member") -- one of two different molecules, having an area on the surface or in a cavity that specifically binds to and is thereby defined as complementary with a particular spatial and polar organization of the other molecule. The members of the specific binding pair are referred to as cognates or as ligand and receptor (antiligand). These may be members of an immunological pair such as antigen-antibody, or may be operator-repressor, nuclease-nucleotide, biotin-avidin, hormones-hormone receptors, nucleic acid duplexes, IgG-protein A, DNA-DNA, DNA-RNA, and the like.

Ligand -- any compound for which a receptor naturally exists or can be prepared.

Receptor ("antiligand") -- any compound or composition capable of recognizing a particular spatial and polar organization of a molecule, e.g., epitopic or determinant site. Illustrative receptors include naturally occurring receptors, e.g., thyroxine binding globulin, antibodies, enzymes, Fab fragments, lectins, nucleic acids, repressors, protection enzymes, protein A, complement component C1q, DNA binding proteins or ligands and the like.

Oligonucleotide Properties:

Potential of an oligonucleotide to hybridize -- the combination of duplex formation rate and duplex dissociation rate that determines the amount of duplex nucleic acid hybrid that will form under a given set of experimental conditions in a given amount of time.

Parameter -- a factor that provides information about the hybridization of an oligonucleotide with a target nucleotide sequence. Generally, the factor is one that is predictive of the ability of an oligonucleotide to hybridize with a target





nucleotide sequence. Such factors include composition factors, thermodynamic factors, chemosynthetic efficiencies, kinetic factors, and the like.

Parameter predictive of the ability to hybridize -- a parameter calculated from a set of oligonucleotide sequences wherein the parameter positively correlates with observed hybridization efficiencies of those sequences. The parameter is, therefore, predictive of the ability of those sequences to hybridize. "Positive correlation" can be rigorously defined in statistical terms. The correlation coefficient $\rho_{x,y}$ of two experimentally measured discreet quantities x and y (N values in each set) is defined as

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$$\rho_{x,y} = \frac{\text{Covariance}(x,y)}{\sqrt{\text{Variance}(x)\text{Variance}(y)}},$$

where the Covariance (x,y) is defined by

15 Covariance $(x, y) = \frac{1}{N} \sum_{j=1}^{N} (x_j - \mu_x) (y_j - \mu_y).$

The quantities μ_x and μ_y are the averages of the quantities x and y, while the variances are simply the squares of the standard deviations (defined below). The correlation coefficient is a dimensionless (unitless) quantity between -1 and 1. A correlation coefficient of 1 or -1 indicates that x and y have a linear relationship with a positive or negative slope, respectively. A correlation coefficient of zero indicates no relationship; for example, two sets of random numbers will yield a correlation coefficient near zero. Intermediate correlation coefficients indicate intermediate degrees of relatedness between two sets of numbers. The correlation coefficient is a good statistical measure of the degree to which one set of numbers predicts a second set of numbers.

Composition factor -- a numerical factor based solely on the composition or sequence of an oligonucleotide without involving additional parameters, such as experimentally measured nearest-neighbor thermodynamic parameters. For instance, the fraction (G+C), given by the formula

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$$f_{GC} = \frac{n_G + n_C}{n_G + n_C + n_A + n_{TorU}},$$

where n_G , n_C , n_A and $n_{Tor\,U}$ are the numbers of G, C, A and T (or U) bases in an oligonucleotide, is an example of a composition factor. Examples of composition factors, by way of illustration and not limitation, are mole fraction (G+C), percent (G+C), sequence complexity, sequence information content, frequency of occurrence of specific oligonucleotide sequences in a sequence database and so forth.

Thermodynamic factor -- numerical factors that predict the behavior of an oligonucleotide in some process that has reached equilibrium. For instance, the free energy of duplex formation between an oligonucleotide and its complement is a thermodynamic factor. Thermodynamic factors for systems that can be subdivided into constituent parts are often estimated by summing contributions from the constituent parts. Such an approach is used to calculate the thermodynamic properties of oligonucleotides.

Examples of thermodynamic factors, by way of illustration and not limitation, are predicted duplex melting temperature, predicted enthalpy of duplex formation, predicted entropy of duplex formation, free energy of duplex formation, predicted melting temperature of the most stable intramolecular structure of the oligonucleotide or its complement, predicted enthalpy of the most stable intramolecular structure of the oligonucleotide or its complement, predicted entropy of the most stable intramolecular structure of the oligonucleotide or its complement, predicted free energy of the most stable intramolecular structure of the oligonucleotide or its complement, predicted melting temperature of the most stable hairpin structure of the oligonucleotide or its complement, predicted enthalpy of the most stable hairpin structure of the oligonucleotide or its complement, predicted entropy of the most stable hairpin structure of the oligonucleotide or its complement, predicted free energy of the most stable hairpin structure of the oligonucleotide or its complement, thermodynamic partition function for intramolecular structure of the oligonucleotide or its complement and the like.

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Chemosynthetic efficiency -- oligonucleotides and nucleotide sequences may both be made by sequential polymerization of the constituent nucleotides. However, the individual addition steps are not perfect; they instead proceed with some fractional efficiency that is less than unity. This may vary as a function of position in the sequence. Therefore, what is really produced is a family of molecules that consists of the desired molecule plus many truncated sequences. These "failure sequences" affect the observed efficiency of hybridization between an oligonucleotide and its complementary target. Examples of chemosynthetic efficiency factors, by way of illustration and not limitation, are coupling efficiencies, overall efficiencies of the synthesis of a target nucleotide sequence or an oligonucleotide probe, and so forth.

Kinetic factor -- numerical factors that predict the rate at which an oligonucleotide hybridizes to its complementary sequence or the rate at which the hybridized sequence dissociates from its complement are called kinetic factors. Examples of kinetic factors are steric factors calculated via molecular modeling or measured experimentally, rate constants calculated via molecular dynamics simulations, associative rate constants, dissociative rate constants, enthalpies of activation, entropies of activation, free energies of activation, and the like.

Predicted duplex melting temperature -- the temperature at which an oligonucleotide mixed with a hybridizable nucleotide sequence is predicted to form a duplex structure (double-helix hybrid) with 50% of the hybridizable sequence. At higher temperatures, the amount of duplex is less than 50%; at lower temperatures, the amount of duplex is greater than 50%. The melting temperature T_m (°C) is calculated from the enthalpy (Δ H), entropy (Δ S) and C, the concentration of the most abundant duplex component (for hybridization arrays, the soluble hybridization target), using the equation

$$T_m = \frac{\Delta H}{\Delta S + R \ln C} - 273.15,$$

where R is the gas constant, 1.987 cal/(mole-°K). For longer sequences (>100 nucleotides), T_m can also be estimated from the mole fraction (G+C), χ_{G+C}, using the equation





$$T_m = 81.5 + 41.0 \chi_{G+C}$$
.

Melting temperature corrected for salt concentration -- polynucleotide duplex melting temperatures are calculated with the assumption that the concentration of sodium ion, Na⁺, is 1 M. Melting temperatures T'_m calculated for duplexes formed at different salt concentrations are corrected via the semi-empirical equation

$$T'_{m}([Na^{+}]) = T_{m} + 16.6 \log([Na^{+}])$$
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Predicted enthalpy, entropy and free energy of duplex formation -- the enthalpy (ΔH), entropy and free energy (ΔG) are thermodynamic state functions, related by the equation

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$$\Delta G = \Delta H - T \Delta S$$
,

where T is the temperature in °K. In practice, the enthalpy and entropy are predicted via a thermodynamic model of duplex formation (the "nearest neighbor" model which is explained in more detail below), and used to calculate the free energy and melting temperature.

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Predicted free energy of the most stable intramolecular structure of an oligonucleotide or its complement -- single-stranded DNA and RNA molecules that contain self-complementary sequences can form intramolecular secondary structures. For instance, the oligonucleotide

25 5'-ACTGGCAATCACAATTGCCAGTAA-3' (SEQ ID NO:1)

can base pair with itself, to form the structure

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where a vertical line indicates Watson-Crick base pair formation. Many such structures are possible for a given sequence; two are of particular interest. The first is the lowest energy "hairpin" structure (formed by folding a sequence back on itself with a connecting loop at least 3 nucleotides long). The second is the lowest energy structure that can be formed by including more complex topologies, such as "bulge loops" (unpaired duplexes between two regions of base-paired duplex) and cloverleaf structures, where 3 base-paired stretches meet at a triple-junction. A good example of a complex secondary structure is the structure of a tRNA molecule, an example of which, namely, yeast tRNA is shown below.

For either type of structure, a value of the free energy of that structure can be calculated, relative to the unpaired strand, by means of a thermodynamic model similar to that used to calculate the free energy of a base-paired duplex structure. Again, the free energy ΔG is calculated from the enthalpy ΔH and the entropy ΔS at a given absolute temperature T via the equation

 $\Delta G = \Delta H - T \Delta S$.

However, in this case there is the added difficulty that the lowest energy structure must be found. For a simple hairpin structure, this optimization can be performed via a relatively simple search algorithm. For more complex structures (such as a cloverleaf) a dynamic programming algorithm, such as that implemented in the program MFOLD, must be used.

Yeast tRNA^{Ala} - The RNA sequence includes many non-standard ribonucleotides, such as D (5,6 dihydrouridine), m¹G (1-methylguanosine), m²G (N²-dimethylguanosine), ψ (pseudouridine), I (inosine), m¹I (1-methylinosine) and T (ribothymidine). Dots (·) mark (non-standard) G=U base pairs. The structure is taken from A. L. Lehninger, *et al.*, <u>Principles of Biochemistry</u>, 2nd Ed. (Worth Publishers, New York, NY, 1993).

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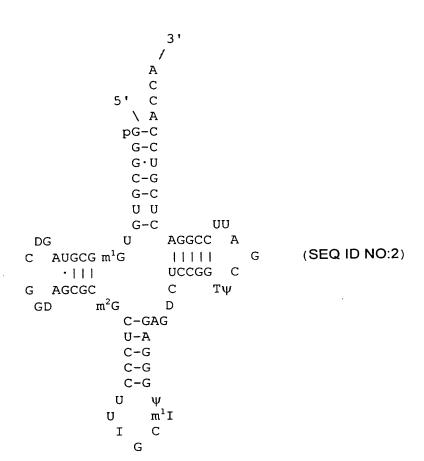
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Coupling efficiencies -- chemosynthetic efficiencies are called coupling efficiencies when the synthetic scheme involves successive attachment of different monomers to a growing oligomer; a good example is oligonucleotide synthesis via phosphoramidite coupling chemistry.

Algorithmic Operations:

Evaluating a parameter -- determination of the numerical value of a numerical descriptor of a property of an oligonucleotide sequence by means of a formula, algorithm or look-up table.

Filter -- a mathematical rule or formula that divides a set of numbers into two subsets. Generally, one subset is retained for further analysis while the other is discarded. If the division into two subsets is achieved by testing the numbers against a simple inequality, then the filter is referred to as a "cut-off". In the context of the current invention, an example by way of illustration and not limitation is the statement "The predicted self structure free energy must be

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greater than or equal to -0.4 kcal/mole," which can be used as a filter for oligonucleotide sequences; this particular filter is also an example of a cut-off.

Filter set -- A set of rules or formulae that successively winnow a set of numbers by identifying and discarding subsets that do not meet specific criteria. In the context of the current invention, an example by way of illustration and not limitation is the compound statement "the predicted self structure free energy must be greater than or equal to -0.4 kcal/mole and the predicted RNA/DNA heteroduplex melting temperature must lie between 60°C and 85°C," which can be used as a filter set for oligonucleotide sequences.

Examining a parameter -- comparing the numerical value of a parameter to some cutoff-value or filter.

Statistical sampling of a cluster -- extraction of a subset of oligonucleotides from a cluster of oligonucleotides based upon some statistical measure, such as rank by oligonucleotide starting position in the sequence complementary to the target sequence.

First quartile, median and third quartile -- If a set of numbers is ranked by value, then the value that divides the lower ¼ from the upper ¾ of the set is the first quartile, the value that divides the set in half is the median and the value that divides the lower ¾ from the upper ¼ of the set is the third quartile.

Poorly correlated -- If it is not possible to perform a "good" prediction, as defined via statistics, of one set of numbers from another set of numbers using a simple linear model, then the two sets of numbers are said to be poorly correlated.

Computer program -- a written set of instructions that symbolically instructs an appropriately configured computer to execute an algorithm that will yield desired outputs from some set of inputs. The instructions may be written in one or several standard programming languages, such as C, C++, Visual BASIC, FORTRAN or the like. Alternatively, the instructions may be written by imposing a template onto a general-purpose numerical analysis program, such as a spreadsheet.

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Experimental System Components:

Small organic molecule -- a compound of molecular weight less than 1500, preferably 100 to 1000, more preferably 300 to 600 such as biotin, fluorescein,

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achieve a consensus behavior. In other words, the oligonucleotide sequences should be sufficiently numerous that several possible probes overlap or fall within a given region that is expected to yield acceptable hybridization efficiency. Since the location of these regions is not known before hand, the best strategy is to equally space the probe sequences along the sequence that is hybridizable to the target sequence. Since regions of acceptable hybridization efficiency are generally on the order of 20 nucleotides in length, a practical strategy is to space the starting nucleotides of the oligonucleotide sequences no more than five basepairs apart. If computation time needed to calculate the predictive parameters is not an issue, then the best strategy is to space the starting nucleotides one nucleotide apart. An important feature of the present invention is to determine oligonucleotides that are clustered along a region of the nucleotide sequence. The individual predictions made for individual oligonucleotide sequences are not very good. However, we have found that the predictions that are experimentally observed tend to form contiguous clusters, while the spurious predictions tend to be solitary. Thus, the number of oligonucleotides should be sufficient to achieve the desired clustering.

Preferably, a set of overlapping sequences is chosen. To this end, the subsequences are chosen so that there is overlap of at least one nucleotide from one oligonucleotide to the next. More preferably, the overlap is two or more nucleotides. Most preferably, the oligonucleotides are spaced one nucleotide apart and the predetermined number is L-N+1 oligonucleotides where L is the length of the nucleotide sequence and N is the length of the oligonucleotides. In the latter situation, the unique oligonucleotides are of identical length N. Thus, a set of overlapping oligonucleotides is a set of oligonucleotides that are subsequences derived from some master sequence by subdividing that sequence in such a way that each subsequence contains either the start or end of at least one other subsequence in the set.

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An example of the above for purposes of illustration and not limitation is presented by the sequence ATGGACTTAGCATTCG (SEQ ID NO:3), from which the following set of overlapping oligonucleotides can be identified:

ATGGACTTAGCA (SEQ ID NO:4)
TGGACTTAGCAT (SEQ ID NO:5)
GGACTTAGCATT (SEQ ID NO:6)
GACTTAGCATTC (SEQ ID NO:7)
ACTTAGCATTCG (SEQ ID NO:8)

In this example the overlapping oligonucleotides are spaced one nucleotide apart. In other words, there is overlap of all but one nucleotide from one oligonucleotide to the next. In the example above, the original nucleotide sequence is 16 nucleotides long (L=16). The length of each of the overlapping oligonucleotides is 12 nucleotides long (N=12) and there are L-N+1 = 5 oligonucleotides.

The length of the oligonucleotides may be the same or different and may vary depending on the length of the nucleotide sequence. The length of the oligonucleotides is determined by a practical compromise between the limits of current chemistries for oligonucleotide synthesis and the need for longer oligonucleotides, which exhibit greater binding affinity for the target sequence and are more likely to occur only once in complicated mixtures of polynucleotide targets. Usually, the length of the oligonucleotides is from about 10 to 50 nucleotides, more usually, from about 25 to 35 nucleotides.

In the next step of the method at least one parameter that is independently predictive of the ability of each of the oligonucleotides of the set to hybridize to the target nucleotide sequence is determined and evaluated for each of the above oligonucleotides. Examples of such a parameter, by way of illustration and not limitation, is a parameter selected from the group consisting of composition factors, thermodynamic factors, chemosynthetic efficiencies, kinetic factors and mathematical combinations of these quantities.

The determination of a parameter may be carried out by known methods. For example, melting temperature of the oligonucleotide/target duplex may be determined using the nearest neighbor method and parameters appropriate for the nucleotide acids involved. For DNA/DNA parameters, see J. SantaLucia Jr., et al., (1996) Biochemistry, 35:3555. For RNA/DNA parameters, see N. Sugimoto, et al., (1995) Biochemistry, 34:11211. Briefly, these methods are

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based on the observation that the thermodynamics of a nucleic acid duplex can be modeled as the sum of a term arising from the entire duplex and a set of terms arising from overlapping pairs of nucleotides ("nearest neighbor" model). For a discussion of the nearest neighbor see J. SantaLucia Jr., et al., (1996)

Biochemistry, supra, and N. Sugimoto, et al., (1995) Biochemistry, supra. For example, the enthalpy ΔH of the duplex formed by the sequence

ATGGACTTAGCA (SEQ ID NO:4)

10 and its perfect complement can be approximated by the equation

$$\begin{split} \Delta H &\cong H_{init} + H_{AT} + H_{TG} + H_{GG} + H_{GA} + H_{AC} \\ &+ H_{CT} + H_{TT} + H_{TA} + H_{AG} + H_{GC} + H_{CA} \; . \end{split}$$

In the above equation, the term H_{init} is the initiation enthalpy for the entire duplex, while the terms H_{AT} , ..., H_{CA} are the so-called "nearest neighbor" enthalpies. Similar equations can be written for the entropy, for the corresponding quantities for RNA homoduplexes, or for DNA/RNA heteroduplexes. The free energy can then be calculated from the enthalpy, entropy and absolute temperature, as described previously.

Predicted free energy of the most stable intramolecular structure of an oligonucleotide (ΔG_{MFOLD}) may be determined using the nucleic acid folding algorithm MFOLD and parameters appropriate for the oligonucleotide, e.g., DNA or RNA. For MFOLD, see J.A. Jaeger, *et al.*, (1989), *supra*. For DNA folding parameters, see J. SantaLucia Jr., *et al.*, (1996), *supra*. Briefly, these methods operate in two steps. First, a map of all possible compatible intramolecular base pairs is made. Second, the global minimum of the free energy of the various possible base pairing configurations is found, using the nearest neighbor model to estimate the enthalpy and entropy, the user input temperature to complete the calculation of free energy, and a dynamic programming algorithm to find the global minimum. The algorithm is computationally intensive; calculation times scale as the third power of the sequence length.





The following Table 1 summarizes groups of parameters that are independently predictive of the ability of each of the oligonucleotides to hybridize to the target nucleotide sequence together with a reference to methods for their determination. Parameters within a given group are known or expected to be strongly correlated to one another, while parameters in different groups are known or expected to be poorly correlated with one another.

Table 1

Group	Parameter	Source or Reference		
	duplex enthalpy, ΔH	Santa Lucia et al., 1996; Sugimoto et al., 1995		
	duplex entropy, ΔS	Santa Lucia et al., 1996; Sugimoto et al., 1995		
	duplex free energy, ΔG	ΔG=ΔH-TΔS (see text)		
	melting temperature, T _m	(see text)		
_	mole fraction (or percent) G+C	self-explanatory		
ı	subsequence duplex enthalpy	Santa Lucia et al., 1996; Sugimoto et al., 1995		
	subsequence duplex entropy	Santa Lucia et al., 1996; Sugimoto et al., 1995		
	subsequence duplex free energy	ΔG=ΔH-TΔS (see text)		
	subsequence duplex T _m	(see text)		
	subsequence duplex mole fraction (or percent) G+C	self-explanatory		
	intramolecular enthalpy, ΔH _{MFOLD}	Jaeger et al., 1989; Santa Lucia et al., 1996		
	intramolecular entropy, ΔS _{MFOLD}	Jaeger et al., 1989; Santa Lucia et al., 1996		
	intramolecular free energy, ΔG _{MFOLD}	ΔG=ΔH-TΔS (see text)		
II	hairpin enthalpy, ∆H _{hairpin}	Jaeger et al., 1989; Santa Lucia et al., 1996		
	hairpin entropy, ΔS _{hairpin}	Jaeger et al., 1989; Santa Lucia et al., 1996		
	hairpin free energy, ΔG _{hairpin}	ΔG=ΔH-TΔS (see text)		
	intramolecular partition function, Z	$Z = \sum_{k \text{ structures}} \exp\left(-\Delta G_{\text{intramolecular}}^{(k)} / RT\right)$		
ll1	sequence complexity	Altschul et al., 1994		
•••	sequence information content	Altschul et al., 1994		
	steric factors	molecular modeling or experiment		
	molecular dynamic simulation	Weber & Hefland, 1979		
IV	enthalpy, entropy & free energy of	measured experimentally		
	activation			
	association & dissociation rates	Patzel & Sczakiel, 1998		
V	oligonucleotide chemosynthetic efficiencies	measured experimentally		
VI	target synthetic efficiencies	measured experimentally		

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In a next step of the present method, a subset of oligonucleotides within the predetermined number of unique oligonucleotides is identified based on the above evaluation of the parameter. A number of mathematical approaches may be followed to sort the oligonucleotides based on a parameter. In one approach a cut-off value is established. The cut-off value is adjustable and can be optimized

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relative to one or more training data sets. This is done by first establishing some metric for how well a cutoff value is performing; for example, one might use the normalized signal observed for each oligonucleotide in the training set. Once such a metric is established, the cutoff value can be numerically optimized to maximize the value of that metric, using optimization algorithms well known to the art. Alternatively, the cutoff value can be estimated using graphical methods, by graphing the value of the metric as a function of one or more parameters, and then establishing cutoff values that bracket the region of the graph where the chosen metric exceeds some chosen threshold value. In essence, the cut off values are chosen so that the rule set used yields training data that maximizes the inclusion of oligonucleotides that exhibit good hybridization efficiency and minimizes the inclusion of oligonucleotides that exhibit poor hybridization efficiency.

A preferred approach to performing such a graph-based optimization of filter parameters is shown in Fig. 3. In Fig. 3, hybridization data from several different genes have been used to prepare a contour plot of relative hybridization intensity as a function of DNA/RNA heteroduplex melting temperature and free energy of the most stable intramolecular structure of the probe. Contours are shown only for regions for which there are data; the white space outside of the outermost contour indicates that there are no experimental data for that region. The details of how the data were obtained can be found in Example 1 below. A summary of the sequences and number of data points employed is shown in Table 2 below. The measured hybridization intensities for each data set were normalized prior to construction of the contour plot depicted in Fig. 3 by dividing each observed intensity by the maximum intensity observed for that gene. In addition, differences in hybridization salt concentrations and hybridization temperatures were accounted for by using the salt concentration-corrected values of the melting temperatures and by subtracting the hybridization temperature from each predicted melting temperature, respectively. The filter set determined by examination of Fig. 3 is indicated by both the dotted open box in the figure and by the inequalities above the box.

One way in which such a contour plot may be prepared involves the use of an appropriate software application such as Microsoft® Excel® or the like. For

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example, the cross-tabulation tool may be used in the Microsoft® Excel® program. Data is accumulated into rectangular bins that are 0.5 kcal ΔG_{MFOLD} wide and 2.5°C T_m wide. In each bin the average values of ΔG_{MFOLD} , $T_m - T_{hyb}$, and the normalized hybridization intensity are calculated. The data is output to the software application DeltaGraph® (Deltapoint, Inc., Monterey, CA) and the contour plot is prepared using the tools and instructions provided.

Table 2

Target (GenBank Accession No.)	Target Strand	No. Data Points	T _{hyb}	[Na [*]] Correction
HIV protease-reverse transcriptase (PRT) ^a (M15654)	Sense	1,022	35°C	-1.4°C
HIV protease-reverse transcriptase (PRT) ^a (M15654)	antisense	1,041	30°C	-1.4°C
HIV protease-reverse transcriptase (PRT) ^b (M15654)	Sense	88	35°C	-1.4°C
Human G3PDH (glyceraldehyde-3- dehydrogenase) ^b (X01677)	antisense	93	35°C	-1.4°C
Human p53 ^b (X02469)	antisense	93	35°C	-1.4°C
Rabbit β-globin ^c (K03256)	antisense	106	30°C	0°C

^a Data from Affymetrix GeneChip™ Array

^b Data from biotinylated probes bound to streptavidin-coated microtiter wells

Once the cut-off value is selected, a subset of oligonucleotides having parameter values greater than or equal to the cut-off value is identified. This refers to the inclusion of oligonucleotides in a subset based on whether the value of a predictive parameter satisfies an inequality.

Examples of identifying a subset of oligonucleotides by establishing cut-off values for predictive parameters are as follows: for melting temperature an inequality might be $60^{\circ}\text{C} \leq T_{m}$; for predicted free energy an inequality, preferably, might be

$$\Delta G_{MFOLD} \ge -0.4 \frac{kcal}{mole}$$
.

In a variation of the above, both a maximum and a minimum cut-off value may be selected. A subset of oligonucleotides is identified whose values fall

^c Literature data: see N. Milner, K. U. Mir & E. M. Southern (1997) Nature Biotech. 15, 537-541.

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within the maximum and minimum values, i.e., values greater than or equal to the minimum cut-off value and less than or equal to the maximum cut-off value. An example of this approach for melting temperature might be the inequality $60^{\circ}C \leq T_{m} \leq 85^{\circ}C$.

With regard to cut off values for T_m the lower limit is most important, and is preferably $T_m = T_{hyb}$, more preferably, $T_m = T_{hyb} + 15^{\circ}C$. The upper cutoff is important when the sequence region under consideration is unusually rich in G and C, and is preferably $T_m = T_{hyb} + 40^{\circ}C$. With regard to ΔG_{MFOLD} the cutoff value is usually greater than or equal to -1.0 kcal/mole. As mentioned above, the cutoff values preferably are determined from real data through experimental observations.

In another approach the parameter values may be converted into dimensionless numbers. The parameter value is converted into a dimensionless number by determining a dimensionless score for each parameter resulting in a distribution of scores having a mean value of zero and a standard deviation of one. The dimensionless score is a number that is used to rank some object (such as an oligonucleotide) to which that score relates. A score that has no units (i.e., a pure number) is called a dimensionless score.

In one approach the following equations are used for converting the values of said parameters into dimensionless numbers:

$$s_{i,x} = \frac{x_i - \langle x \rangle}{\sigma_{\{x\}}},\,$$

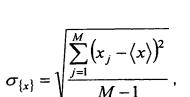
where $s_{i,x}$ is the dimensionless score derived from parameter x calculated for oligonucleotide i, x_i is the value of parameter x calculated for oligonucleotide i, x_i is the average of parameter x calculated for all of the oligonucleotides under consideration for a given nucleotide sequence target, and $\sigma_{(x)}$ is the standard deviation of parameter x calculated for all of the oligonucleotides under consideration for a given nucleotide sequence target, and is given by the equation

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where M is the number of oligonucleotides. The resulting distribution of scores, {s} has a mean value of zero and a standard deviation of one. These properties can be important for a combination of the scores discussed below.

The use of a dimensionless number approach may further include calculating a combination score S_i by evaluating a weighted average of the individual values of the dimensionless scores $s_{i,x}$ by the equation:

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$$S_i = \sum_{\{x\}} q_x \, s_{i,x}$$
,

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where q_x is the weight assigned to the score derived from parameter x, the individual values of q_x are always greater than zero, and the sum of the weights q_x is unity.

In another variation of the above approach, the method of calculation of the composite parameter is optimized based on the correlation of the individual composite scores to real data, as explained more fully below.

In one approach the calculation of the composite score further involves determining a moving window-averaged combination score $\langle S_i \rangle$ for the *i*th probe by the equation:

$$\langle S_i \rangle = \frac{1}{w} \sum_{j=i-\frac{w-1}{2}}^{i+\frac{w-1}{2}} S_j$$
, $w = \text{an odd integer}$,

where w is the length of the window for averaging (i.e., w nucleotides long), and then applying a cutoff filter to the value of $\langle S_i \rangle$. This procedure results in smoothing (smoothing procedure) by turning each score into a consensus metric for a set of w adjacent oligonucleotide probes. The score, referred to as the "smoothed score," is essentially continuous rather than a few discrete values. The

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value of the smoothed score is strongly influenced by clustering of scores with high or low values; window averaging therefore provides a measurement of cluster size.

An advantage of the dimensionless score approach to the probe prediction algorithm is that it is easy to objectively optimize. In one approach to training the algorithm, optimization of the weights q_x above may be performed by varying the values of the weights so that the correlation coefficient $\rho_{\{< S_i>\},\{V_i\}}$ between the set of window-averaged combination scores $\{< S_i>\}$ and a set of calibration experimental measurements $\{V_i\}$ is maximized. The correlation coefficient $\rho_{\{< S_i>\},\{V_i\}}$ is calculated from the equation

$$\rho_{\{\langle S_i \rangle\}, \{V_i\}} = \left(\frac{1}{M}\right) \frac{\text{Covariance}(\langle S \rangle, V)}{\sigma_{\{\langle S_i \rangle\}} \sigma_{\{V_i\}}} .$$

where M is the number of window averaged, combination dimensionless scores and the number of corresponding measurements, the covariance is as defined earlier (see earlier equations) and $\sigma_{\{\langle S_i \rangle\}}$ and $\sigma_{\{V_i\}}$ are the standard deviations of $\{\langle S_i \rangle\}$ and $\{V_i\}$, as defined previously. An example of this approach is shown in Example 2, below.

In another approach the parameter is derived from one or more factors by mathematical transformation of the factors. This involves the calculation of a new predictive parameter from one or more existing predictive parameters, by means of an equation. For instance, the equilibrium constant K_{open} for formation of an oligonucleotide with no intramolecular structure from its structured form can be calculated from the intramolecular structure free energy ΔG_{MFOLD} , using the equation:

$$K_{open} = \exp\left(\frac{\Delta G_{MFOLD}}{RT}\right).$$

In a next step of the method oligonucleotides in the subset are then identified that are clustered along a region of the nucleotide sequence that is

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hybridizable to the target nucleotide sequence. For example, consider a set of overlapping oligonucleotides identified by dividing a nucleotide sequence into subsequences. A subset of the oligonucleotides is obtained as described above. In general, this subset is obtained by applying a rule that rejects some members of the set. For the remaining members of the set, namely, the subset, there will be some average number of nucleotides in the nucleotide sequence between the first nucleotides of adjacent remaining subsequences. If, for some sub-region of the nucleotide sequence, the average number of nucleotides in the nucleotide sequence between the first nucleotides of adjacent remaining subsequences is less than the average for the entire nucleotide sequence, then the oligonucleotides are clustered. The smaller the average number of nucleotides between the first nucleotides of adjacent oligonucleotides, the stronger the clustering. The strongest clustering occurs when there are no intervening nucleotides between adjacent starting nucleotides. In this case, the oligonucleotides are said to be contiguous and may be referred to as contiguous sequence elements or "contigs."

Accordingly, in this step oligonucleotides are sorted based on length of contiguous sequence elements. Oligonucleotides in the subset determined above are identified that are contiguous along a region of the input nucleic acid sequence. The length of each contig that is equal to the number of oligonucleotides in each contig, namely, oligonucleotides from the above step whose complement begin at positions m+1, m+2,, m+k in the target sequence, form a contig of length k. Contigs can be identified and contig length can be calculated using, for example, a Visual Basic ® module that can be incorporated into a Microsoft ® Excel workbook.

Cluster size can be defined in several ways:

For contiguous clusters, the size is simply the number of adjacent oligonucleotides in the cluster. Again, this may also be referred to as contiguous sequence elements. The number may also be referred to as "contig length". For example, consider the nucleotide sequence discussed above, namely, ATGGACTTAGCATTCG (SEQ ID NO:3) and the identified set of overlapping oligonucleotides

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rhodamine and other dyes, tetracycline and other protein binding molecules, and haptens, *etc*. The small organic molecule can provide a means for attachment of a nucleotide sequence to a label or to a support.

Support or surface -- a porous or non-porous water insoluble material. The surface can have any one of a number of shapes, such as strip, plate, disk, rod, particle, including bead, and the like. The support can be hydrophilic or capable of being rendered hydrophilic and includes inorganic powders such as glass, silica, magnesium sulfate, and alumina; natural polymeric materials, particularly cellulosic materials and materials derived from cellulose, such as fiber containing papers, e.g., filter paper, chromatographic paper, etc.; synthetic or modified naturally occurring polymers, such as nitrocellulose, cellulose acetate, poly (vinyl chloride), polyacrylamide, cross linked dextran, agarose, polyacrylate, polyethylene, polypropylene, poly(4-methylbutene), polystyrene, polymethacrylate, poly(ethylene terephthalate), nylon, poly(vinyl butyrate), etc.; either used by themselves or in conjunction with other materials; glass available as Bioglass, ceramics, metals, and the like. Natural or synthetic assemblies such as liposomes, phospholipid vesicles, and cells can also be employed.

Binding of oligonucleotides to a support or surface may be accomplished by well-known techniques, commonly available in the literature. See, for example, A. C. Pease, *et al.*, Proc. Nat. Acad. Sci. USA, 91:5022-5026 (1994).

Label -- a member of a signal producing system. Usually the label is part of a target nucleotide sequence or an oligonucleotide probe, either being conjugated thereto or otherwise bound thereto or associated therewith. The label is capable of being detected directly or indirectly. Labels include (i) reporter molecules that can be detected directly by virtue of generating a signal, (ii) specific binding pair members that may be detected indirectly by subsequent binding to a cognate that contains a reporter molecule, (iii) oligonucleotide primers that can provide a template for amplification or ligation or (iv) a specific polynucleotide sequence or recognition sequence that can act as a ligand such as for a repressor protein, wherein in the latter two instances the oligonucleotide primer or repressor protein will have, or be capable of having, a reporter molecule. In general, any reporter molecule that is detectable can be used.

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The reporter molecule can be isotopic or nonisotopic, usually non-isotopic, and can be a catalyst, such as an enzyme, a polynucleotide coding for a catalyst, promoter, dye, fluorescent molecule, chemiluminescent molecule, coenzyme, enzyme substrate, radioactive group, a small organic molecule, amplifiable polynucleotide sequence, a particle such as latex or carbon particle, metal sol,

be a fluorescent group such as fluorescein, a chemiluminescent group such as luminol, a terbium chelator such as N-(hydroxyethyl) ethylenediaminetriacetic acid that is capable of detection by delayed fluorescence, and the like.

crystallite, liposome, cell, etc., which may or may not be further labeled with a dye, catalyst or other detectable group, and the like. The reporter molecule can

The label is a member of a signal producing system and can generate a detectable signal either alone or together with other members of the signal producing system. As mentioned above, a reporter molecule can be bound directly to a nucleotide sequence or can become bound thereto by being bound to an sbp member complementary to an sbp member that is bound to a nucleotide sequence. Examples of particular labels or reporter molecules and their detection can be found in U.S. Patent No. 5,508,178 issued April 16, 1996, at column 11, line 66, to column 14, line 33, the relevant disclosure of which is incorporated herein by reference. When a reporter molecule is not conjugated to a nucleotide sequence, the reporter molecule may be bound to an sbp member complementary to an sbp member that is bound to or part of a nucleotide sequence.

Signal Producing System -- the signal producing system may have one or more components, at least one component being the label. The signal producing system generates a signal that relates to the presence or amount of a target polynucleotide in a medium. The signal producing system includes all of the reagents required to produce a measurable signal. Other components of the signal producing system may be included in a developer solution and can include substrates, enhancers, activators, chemiluminescent compounds, cofactors, inhibitors, scavengers, metal ions, specific binding substances required for binding of signal generating substances, and the like. Other components of the signal producing system may be coenzymes, substances that react with enzymic products, other enzymes and catalysts, and the like. The signal producing system

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provides a signal detectable by external means, by use of electromagnetic radiation, desirably by visual examination. Signal-producing systems that may be employed in the present invention are those described more fully in U.S. Patent No. 5,508,178, the relevant disclosure of which is incorporated herein by reference.

Ancillary Materials -- Various ancillary materials will frequently be employed in the methods and assays utilizing oligonucleotide probes designed in accordance with the present invention. For example, buffers and salts will normally be present in an assay medium, as well as stabilizers for the assay medium and the assay components. Frequently, in addition to these additives, proteins may be included, such as albumins, organic solvents such as formamide, quaternary ammonium salts, polycations such as spermine, surfactants, particularly non-ionic surfactants, binding enhancers, e.g., polyalkylene glycols, or the like.

DETAILED DESCRIPTION OF THE INVENTION

The invention is directed to methods or algorithms for predicting oligonucleotides specific for a nucleic acid target where the oligonucleotides exhibit a high potential for hybridization. The algorithm uses parameters of the oligonucleotide and the oligonucleotide/target nucleotide sequence duplex, which can be readily predicted from the primary sequences of the target polynucleotide and candidate oligonucleotides. In the methods of the present invention, oligonucleotides are filtered based on one or more of these parameters, then further filtered based on the sizes of clusters of oligonucleotides along the input polynucleotide sequence. The methods or algorithms of the present invention may be carried out using either relatively simple user-written subroutines or publicly available stand-alone software applications (e.g., dynamic programming algorithm for calculating self-structure free energies of oligonucleotides). The parameter calculations may be orchestrated and the filtering algorithms may be implemented using any of a number of commercially available computer programs as a framework such as, e.g., Microsoft® Excel spreadsheet, Microsoft® Access relational database and the like. The basic steps involved in the present methods involve parsing a sequence that is complementary to a target nucleotide sequence into a set of overlapping oligonucleotide sequences, evaluating one or more

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parameters for each of the oligonucleotide sequences, said parameter or parameters being predictive of probe hybridization to the target nucleotide sequence, filtering the oligonucleotide sequences based on the values for each parameter, filtering the oligonucleotide sequences based on the length of contiguous sequence elements and ranking the contiguous sequence elements based on their length. We have found that oligonucleotides in the longest contiguous sequence elements generally show the highest hybridization efficiencies.

The present methods are based on our recognition that oligonucleotides showing high hybridization efficiencies tend to form clusters. It is believed that this clustering reflects local regions of the target nucleotide sequence that are unstructured and accessible for oligonucleotide binding. Oligonucleotides that are contiguous along a region of the input nucleic acid sequence are identified. These oligonucleotides are sorted based on the length of the contiguous sequence elements. The sorting approach used in the present invention apparently serves as a surrogate for the calculation of local secondary structure of the target nucleotide sequence. This is supported by our observation that treatments intended to eliminate long-range nucleic acid structure (e.g., random fragmentation) do not eliminate the differences in hybridization yields across oligonucleotide probe arrays. This implies that major determinants of efficient hybridization are local regions of the target sequence. The identification of contiguous sequence elements is a simple and efficient method for recognizing clusters of such determinants and, thus, for identifying oligonucleotide probes that exhibit high hybridization efficiency for a target nucleotide sequence.

As mentioned above one embodiment of the present invention is a method for predicting the potential of an oligonucleotide to hybridize to a target nucleotide sequence. A predetermined number of unique oligonucleotides is identified. The length of the oligonucleotides may be the same or different. The oligonucleotides are unique in that no two of the oligonucleotides are identical. The unique oligonucleotides are chosen to sample the entire length of a nucleotide sequence that is hybridizable with the target nucleotide sequence. The actual number of oligonucleotides is generally determined by the length of the nucleotide sequence and the desired result. The number of oligonucleotides should be sufficient to

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ATGGACTTAGCA (SEQ ID NO:4)
TGGACTTAGCAT (SEQ ID NO:5)
GGACTTAGCATT (SEQ ID NO:6)
GACTTAGCATTC (SEQ ID NO:7)
ACTTAGCATTCG (SEQ ID NO:8)

Suppose that, after calculation and evaluation of the predictive parameters, four nucleotides remain:

ATGGACTTAGCA TGGACTTAGCAT GGACTTAGCATT	(SEQ ID NO:4) (SEQ ID NO:5) (SEQ ID NO:6)	contig
ACTTAGCATTCG	(SEQ ID NO:8)	single oligonucleotide

A "contig" encompassing three of the oligonucleotides of the subset is present together with a single oligonucleotide. The contig length is 3 oligonucleotides.

Alternatively, cluster size at some position in the sequence hybridizable or complementary to the target sequence may be defined as the number of oligonucleotides whose center nucleotides fall inside a region of length M centered about the position in question, divided by M. This definition of clustering allows small gaps in clusters. In the example used above for contiguous clusters, if M was 10, then the cluster size would step through the values 0/10,..., 0/10, 1/10, 2/10, 3/10, 3/10, 4/10, 4/10, 4/10, 4/10, 4/10, 3/10, 2/10, 1/10, 1/10, 0/10 as the center of the window of length 10 passed through the cluster. In each fraction, the numerator is the number of oligonucleotide sequences that have satisfied the filter set and whose central nucleotides are within a window 10 nucleotides long, centered about the nucleotide under consideration. The denominator (10) is simply the window length.

Another alternative is to define the size of a cluster at some position in the sequence hybridizable or complementary to the target sequence as the number of oligonucleotide sequences overlapping that position. This definition is equivalent to the last definition with M set equal to the oligonucleotide probe length and omission of the division by M.

Finally, cluster size can be approximated at each position in a nucleotide sequence by dividing the sequence into oligonucleotides, evaluating a numerical score for each oligonucleotide, and then averaging the scores in the neighborhood

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its neighbors.



of each position by means of a moving window average as described above.

Window averaging has the effect of reinforcing clusters of high or low values around a particular position, while canceling varying values about that position.

The window average, therefore, provides a score that is sensitive to both the hybridization potential of a given oligonucleotide and the hybridization potentials of

In a next step of the present method, the oligonucleotides in the subset are ranked. Generally, this ranking is based on the lengths of the clusters or contigs, sizes of the clusters or values of a window averaged score. Oligonucleotides found in the longest contigs or largest clusters, or possessing the highest window averaged scores usually show the highest hybridization efficiencies. Often, the highest signal intensity within the cluster corresponds to the median oligonucleotide of the cluster. However, the peak signal intensity within the contig can be determined experimentally, by sampling the cluster at its first quartile, midpoint and third quartile, measuring the hybridization efficiencies of the sampled oligonucleotides, interpolating or extrapolating the results, predicting the position of the optimal probe, and then iterating the probe design process.

Fig. 1 shows a diagram of an example of the above-described method by way of illustration and not limitation. Referring to Fig. 1 a target sequence of length L from, e.g., a database, is used to generate a sequence that is hybridizable to the target sequence from which candidate oligonucleotide probe sequences are generated. One or more parameters are calculated for each of the oligonucleotide probe sequences. The candidate oligonucleotide probe sequences are filtered based on the values of the parameters. Clustering of the filtered candidate probe sequences is evaluated and the clusters are ranked by size. Then, the oligonucleotide probes are statistically sampled and synthesized. Further evaluation may be made by evaluating the hybridization of the selected oligonucleotide probes in real hybridization experiments. The above process may be reiterated to further define the selection. In this way only a small fraction of the potential oligonucleotide probe candidates are synthesized and tested. This is in sharp contrast to the known method of synthesizing and testing all or a major portion of potential oligonucleotide probes for a given target sequence.

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The methods of the present invention are preferably carried out at least in part with the aid of a computer. For example, an IBM® compatible personal computer (PC) may be utilized. The computer is driven by software specific to the methods described herein.

The preferred computer hardware capable of assisting in the operation of the methods in accordance with the present invention involves a system with at least the following specifications: Pentium® processor or better with a clock speed of at least 100 MHz, at least 32 megabytes of random access memory (RAM) and at least 80 megabytes of virtual memory, running under either the Windows 95 or Windows NT 4.0 operating system (or successor thereof).

As mentioned above, software that may be used to carry out the methods may be either Microsoft Excel or Microsoft Access, suitably extended via user-written functions and templates, and linked when necessary to stand-alone programs that calculate specific parameters (e.g., MFOLD for intramolecular thermodynamic parameters). Examples of software programs used in assisting in conducting the present methods may be written, preferably, in Visual BASIC, FORTRAN and C++, as exemplified below in the Examples. It should be understood that the above computer information and the software used herein are by way of example and not limitation. The present methods may be adapted to other computers and software. Other languages that may be used include, for example, PASCAL, PERL or assembly language.

Fig. 2 depicts a more specific approach to a method in accordance with the present invention. Referring to Fig. 2, a sequence of length L is obtained from a database such as GenBank, UniGene or a proprietary sequence database. Probe length N is determined by the user based on the requirements for sensitivity and specificity and the limitations of the oligonucleotide synthetic scheme employed. The probe length and sequence length are used to generate L-N+1 candidate oligonucleotide probes, i.e., from every possible starting position. An initial selection is made based on local sequence predicted thermodynamic properties. To this end, melting temperature T_m and the self-structure free energy ΔG_{MFOLD} , are calculated for each of the potential oligonucleotide probe: target nucleotide sequence complexes. Next, M probes that satisfy T_m and ΔG_{MFOLD} filters are selected. A further selection can be made based on clustering of "good"

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parameters. Good parameters are parameters that satisfy all of the filters in the filter set. Clustering is defined by any of the methods described previously; in Fig. 2, the "contig length" definition of clustering is used.

For each of the M oligonucleotide sequences that satisfied all filters the question is asked whether the oligonucleotide sequence immediately following the sequence under consideration is also one of the sequences that satisfied all of the filters. If the answer to this question is NO, then one stores the current value of the contig length counter, resets the counter to zero and proceeds to the next oligonucleotide sequence that satisfied all filters. If the answer to the question is YES, then 1 is added to the contig length counter and, if the counter now equals 1 (i.e., this is the first oligonucleotide probe sequence in the contig), the starting position of the oligonucleotide is stored. One then moves to the next oligonucleotide that satisfied all filters, which, in this case, is the same as the next oligonucleotide before the application of the filter set. The process is repeated until all M filtered oligonucleotide sequences have been examined. In this way, a single pass through the set of M filtered oligonucleotide sequences generates the lengths and starting positions of all contigs.

Next, contigs are ranked based on the lengths of their contiguous sequence elements. Longer contig lengths generally correlate with higher hybridization efficiencies. All oligonucleotides of the higher-ranking contigs may be considered, or candidate oligonucleotide probes may be picked. For example, candidate oligonucleotide probes can be picked one quarter, one half and three quarters of the way through each contig. The latter approach provides local curvature determination after experimental determination of hybridization efficiencies, which allows either interpolation or extrapolation of the positions of the next probes to be synthesized in order to close in on the optimal probe in the region. If the contig brackets the actual peak of hybridization efficiency, the process will converge in 2-3 iterations. If the contig lies to one side of the actual peak, the process will converge in 3-4 iterations.

The above illustrative approach is further described with reference to the following DNA nucleotide sequence, which is the complement of the target RNA nucleotide sequence:

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GTCCAAAAAGGGTCAGTCTACCTCCCGCCATAAAAAACTCATGTTCAAGA (SEQ ID NO:9).

In the first step of the method, the nucleotide sequence is divided into overlapping oligonucleotides that are 25 nucleotides in length. This length is chosen because it is an effective compromise between the need for sensitivity (enhanced by longer oligonucleotides) and the chemosynthetic efficiency of schemes for synthesis of surface-bound arrays of oligonucleotide probes.

Next, the estimated duplex melting temperatures (T_m) and self-structure free energies (ΔG_{MFOLD}) are calculated for each oligonucleotide in the set of overlapping oligonucleotides. The values are obtained from a user-written function that calculates DNA/RNA heteroduplex thermodynamic parameters (see N. Sugimoto, et al., Biochemistry, 34:11211 (1995)) and a modified version of the program MFOLD that estimates the free energy of the most stable intramolecular





structure of a single stranded DNA molecule (see J.A. Jaeger, *et al.*, (1989), *supra*, respectively. The steps are illustrated below.

	5	GTCCAAAAAGGGTCAGTCTACCTCCCGCCATAAAAAACTCATGTTCAAGA	(target cor	nplement	sequence)
			T _m (°C)	ΔG_{MFOI}	D
		GTCCAAAAAGGGTCAGTOTACCTCC	71.77	-1.20	SEQ ID NO:10
		TCCAAAAAGGGTCAGTCAACCTCCC	71.99	-1.20	SEQ ID NO:11
	10	CCAAAAAGGGTCAGTCTACCTCCCG	70.78	-1.20	SEQ ID NO:12
		CAAAAAGGGTCAGTCTAGCTCCCGC	71.23	-1.20	SEQ ID NO:13
		AAAAAGGGTCAGTCTACCTCCCGCC	73.07	-1.20	SEQ ID NO:14
		AAAAGGGTCAGTCTACCTCCCGCCA	75.68	-1.20	SEQ ID NO:15
		AAAGGGTCAGTCTACCTCCCGCCAT	77.53	-1.20	SEQ ID NO:16
	15	AAGGGTCAGTCTACCTOCCGCCATA	79.03	-1.20	SEQ ID NO:17
		AGGGTCAGTCTACCTC Q CGCCATAA	79.03	-1.20	SEQ ID NO:18
- 1	A21	gggtcagtctacctcc t gccataaa	76.85	-1.20	SEQ ID NO:19
SUP	A2/	ggtcagtctacctccc ccataaaa	73.10	-0.80	SEQ ID NO:20
		GTCAGTCTACCTCCCGCCATAAAAA	69.50	0.90	SEQ ID NO:21
	20	tcagtctacctcccgdcataaaaaa	65.60	0.90	SEQ ID NO:22
14 <u></u> §		CAGTCTACCTCCCGCOATAAAAAAC	64.96	0.90	SEQ ID NO:23
		agtctacctcccgcc \ taaaaaact	65.	1.10	SEQ ID NO:24
Territi gene		GTCTACCTCCCGCCA † AAAAAACTC	66.36	2.40	SEQ ID NO:25
		TCTACCTCCCGCCATAAAAACTCA	64.97	2.90	SEQ ID NO:26
<u>U</u> 1	25	CTACCTCCGCCATA\AAAACTCAT	63.96	2.70	SEQ ID NO:27
190		tacctcccgccata A aaaactcatg	62.58	1.10	SEQ ID NO:28
क्ष्मीतः इत्या		acctcccgccataa A aaactcatgt	65.10	0.40	SEQ ID NO:29
=		CCTCCCGCCATAAAAAACTCATGTT	64.96	0.10	SEQ ID NO:30
		CTCCCGCCATAAAA A ACTCATGTTC	63.37	-0.10	SEQ ID NO:31
100 E	30	TCCCGCCATAAAAAAACTCATGTTCA	62.86	-0.10	SEQ ID NO:32
: 1±3 : :		CCCGCCATAAAAAAÇTCATGTTCAA	60.47	-0.10	SEQ ID NO:33
<u></u>		CCGCCATAAAAAAC <mark>T</mark> CATGTTCAAG	57.98	-0.10	SEQ ID NO:34
7		cgccataaaaaact c atgttcaaga	56.20	-0.10	SEQ ID NO:35
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56.20

-0.10

Next, the oligonucleotide sequences are filtered on the basis of T_m . A high and low cut-off value may be selected, for example, $60^{\circ}C \leq T_m \leq 85^{\circ}C$. Thus, oligonucleotides having T_m values falling within the above range are retained. Those outside the range are discarded, which is indicated below by lining out of those oligonucleotides and parameter values.

		GTCCAAAAAGGGTCAGTCTACCTCCCGCCATAAAAAACTCATGTTCAAGA	(target cor	nplement sequence)
	10		T _m (°C)	ΔG_{MFOLD}
		GTCCAAAAAGGGTCAGTCTACCTCC	71.77	-1.20
		TCCAAAAAGGGTCAGTCTACCTCCC	71.99	-1.20
		CCAAAAAGGGTCAGTCTACCTCCCG	70.78	-1.20
		CAAAAAGGGTCAGTCTACCTCCCGC	71.23	-1.20
	15	AAAAAGGGTCAGTCTACCTCCCGCC	73.07	-1.20
	,	AAAAGGGTCAGTCTACCTCCCGCCA	75.68	-1.20
ī	\	AAAGGGTCAGTCTACCTCCCGCCAT	77.53	-1.20
	A21	AAGGGTCAGTCTACCTCCCGCCATA	79.03	-1.20
ENI!		AGGGTCAGTCTACCTCCCGCCATAA	79.03	-1.20
II - 6	~20 (GGGTCAGTCTACCTCCCGCCATAAA	76.85	-1.20
_ (A)	, ,	GGTCAGTCTACCTCCCGCCATAAAA	73.10	-0.80
		GTCAGTCTACCTCCCGCCATAAAAA	69.50	0.90
달 :		TCAGTCTACCTCCCGCCATAAAAAA	65.60	0.90
~_j		CAGTCTACCTCCCGCCATAAAAAAC	64.96	0.90
	25	AGTCTACCTCCCGCCATAAAAAACT	65.48	1.10
æ		GTCTACCTCCCGCCATAAAAAACTC	66.36	2.40
		TCTACCTCCCGCCATAAAAAACTCA	64.97	2.90
		CTACCTCCCGCCATAAAAAACTCAT	63.96	2.70
IJ		TACCTCCCGCCATAAAAAACTCATG	62.58	1.10
	30	ACCTCCCGCCATAAAAAACTCATGT	65.10	0.40
LT		CCTCCCGCCATAAAAAACTCATGTT	64.96	0.10
		CTCCCGCCATAAAAACTCATGTTC	63.37	-0.10
		TCCCGCCATAAAAACTCATGTTCA	62.86	-0.10
<u>La</u>		CCCGCCATAAAAAACTCATGTTCAA	60.47	-0.10
	35	CCCCATAAAAAACTCATCTTCAAC	57.98	-0.10

CCCCATAAAAAACTCATCTTCAACA





Next, the oligonucleotide sequences remaining after the above exercise are filtered on the basis of ΔG_{MFOLD} and are retained if the value is greater than - 0.4. Those oligonucleotides with a ΔG_{MFOLD} less than - 0.4 are discarded, which is indicated below by double lining out of those oligonucleotides and parameter values.

	GTCCAAAAAGGGTCAGTCTACCTCCCGCCATAAAAAACTCATGTTCAAGA	(target cor	nplement sequence)
10			
		T _m (°C)	ΔG_{MFOLD}
	CTCCAAAAACCCTCAACTCTACCTCC	71.77	- 1.20
	TCCANANGCCTCACTCTACCTCCC	71.99	- 1.20
4	COMMANGEET CACTETACCT CCCC	70.78	- 1.20
15	GAAAAAGCCTCACTCTACCTCCCCC	71.23	- 1.20
	AAAAACCCTCACTCTACCTCCCCCCC	73.07	- 1.20
	AAAACCCTCACTCTACCTCCCCCCA	75.68	- 1-20
	PARCCETCRETTRECTECCECAT	77.53	- 1.20
	AACCCTCACTCTACCTCCCCCCATA	79.03	- 1-20
20	ACCCTCACTCTCCCCCCGATAA	79.03	- 1.20
_	CECTCACTCTACCTCCCCCATAAA	76.85	~ 1+20
a A	CGTGAGTGTAGGTGGGGGATAAAA	73.10	- 0.80
(D) "	GTCAGTCTACCTCCCGCCATAAAAA	69.50	0.90
حريب	TCAGTCTACCTCCCGCCATAAAAAA	65.60	0.90
⁻ 25	CAGTCTACCTCCCGCCATAAAAAAC	64.96	0.90
	AGTCTACCTCCCGCCATAAAAAACT	65.48	1.10
	GTCTACCTCCCGCCATAAAAAACTC	66.36	2.40
	TCTACCTCCCGCCATAAAAAACTCA	64.97	2.90
00	CTACCTCCCGCCATAAAAAACTCAT	63.96	2.70
30	TACCTCCCGCCATAAAAAACTCATG	62.58	1.10
	ACCTCCCGCCATAAAAAACTCATGT	65.10	0.40
	CCTCCCGCCATAAAAAACTCATGTT	64.96	0.10
	CTCCCGCCATAAAAACTCATGTTC	63.37	-0.10
~=	TCCCGCCATAAAAACTCATGTTCA	62.86	-0.10
35	CCCGCCATAAAAAACTCATGTTCAA	60.47	-0.10
	CCGCCATAAAAACTCATGTTCAAG	57.98	-0.10
	000000000000000000000000000000000000000		

CCCCATAAAAAACTCATGTTCAAGA

-0.10





Clusters of retained oligonucleotides are identified and ranked based on cluster size. In this example, a contiguous cluster of 13 retained oligonucleotides is identified by the vertical black bar on the left. Any or all of the oligonucleotides in this cluster may be evaluated experimentally.

		GTCCAAAAAGGGTCAGTCTACCTCCCGCCATAAAAAACTCATGTTCAAGA	(target cor	mplement sequence
	10		T _m (°C)	ΔG_{MFOLD}
		CTCCAAAAACCCTCACTCTACCTCC	71.77	- 1.20
		TGCNANACCGTCACTCTACCTCCCC	71.99	- 1-20
		GGAAAAAGGCTCACTCTAGGCGGC	70.78	- 1-20
	15	CAAAAAGCCTCACTCTACCTCCCCC	71.23	- 1 -20
	15	**************************************	73.07	- 1.20
		AMMAGGETCACTCTACGTCCCCCCA	75.68	- 1.20
	- 1	17 / AMAGGETGAGTGTAGGTGGGGGAT	77.53	- 1-20
,	SUB F	AAGCTCACTCTACCTCCCCCATA	79.03	- 1.20
آسية عد	ر مور	AGGCTGAGTGTAGGTGGGCCCATAA	79.03	- 1.20
قيق	20	CECTCACTCTCCCCCCATAAA	76.85	- 1.20
	\mathcal{O}^{\prime}	CCTCACTCTCCCCCCATAAAA	73.10	- 0.80
Ø		GTCAGTCTACCTCCCGCCATAAAAA	69.50	0.90
المطاع المريدي		TCAGTCTACCTCCCGCCATAAAAA	65.60	0.90
F	25	CAGTCTACCTCCCGCCATAAAAAAC	64.96	0.90
	25	AGTCTACCTCCCGCCATAAAAAACT	65.48	1.10
		GTCTACCTCCCGCCATAAAAAACTC	66.36	2.40
ट्यांट्रीसाट रेडाक्ट		TCTACCTCCCGCCATAAAAAACTCA	64.97	2.90
-		CTACCTCCCGCCATAAAAAACTCAT	63.96	2.70
=	0.0	TACCTCCCGCCATAAAAAACTCATG	62.58	1.10
Ū	30	ACCTCCCGCCATAAAAAACTCATGT	65.10	0.40
Ŋ		CCTCCCGCCATAAAAAACTCATGTT	64.96	0.10
į.		CTCCCGCCATAAAAAACTCATGTTC	63.37	-0.10
		TCCCGCCATAAAAAACTCATGTTCA	62.86	-0.10
U		CCCGCCATAAAAAACTCATGTTCAA	60.47	-0.10
	35	CCCCCATAAAAAACTCATCTTCAAC	57.98	-0.10
		CCCCATAAAAAACTCATGTTCAAGA	56.20	-0.10

Alternatively, in one approach the oligonucleotides at the first quartile, the median and the third quartile of the cluster may be selected for experimental evaluation, indicated below by bold print.

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		GTCCAAAAAGGGTCAGTCTACCTCCCGCCATAAAAAACTCATGTTCAAGA	(target con	nplement sequence)
		\	T _m (°C)	∆G _{MFOLD}
	10	GTGGANANACCGTGACGTGGGGGGGGGGGGGGGGGGGGGG	71.77	- 1.20
		#CCAAAAACGC#CACTCTACCTCCC	71.99	- 1 - 20
		GCANANAGGGTCAGTGTAGGTGGGG	70.78	- 1.20
		CANANACCTCACTCTACCTCCCCC	71.23	- 1.20
- 1	ALI	AAAAAGCCTCACTCTACCTCCCCCCCCCCCCCCCCCCCC	73.07	- 1.20
SVP	15	ANNACCTCACTCTACCTCCCCCCA	75.68	- 1+20
- CM	المقدر	AAACCCTCACTCTACCTCCCCCCAT	77.53	- 1.20
	•	AACCCTCACTCTACCTCCCCCCATA	79.03	- 1.20
		ACCCTCACTCTACCTCCCCCCATAA	79.03	- 1+20
		GGGTGAGTGTAGGTGGGGGGGATAAA	76.85	- 1-20
	20	CCTCACTCTACCTCCCCCATAAAA	73.10	- 0.80
J		GTCAGTCTACCTCCCGCCATAAAAA \	69.50	0.90
<u>%5553</u> 5-, 9		TCAGTCTACCTCCCGCCATAAAAAA	65.60	0.90
		CAGTCTACCTCCCGCCATAAAAAAC	64.96	0.90
Ţ		AGTCTACCTCCCGCCATAAAAAACT	65.48	1.10
	25	GTCTACCTCCCGCCATAAAAAACTC \	66.36	2.40
		TCTACCTCCCGCCATAAAAACTCA \	64.97	2.90
		CTACCTCCCGCCATAAAAACTCAT \	63.96	2.70
		TACCTCCCGCCATAAAAAACTCATG \	62.58	1.10
		ACCTCCCGCCATAAAAAACTCATGT \	65.10	0.40
Ħ	30	CCTCCCGCCATAAAAAACTCATGTT \	64.96	0.10
		CTCCCGCCATAAAAACTCATGTTC \	63.37	-0.10
711		TCCCGCCATAAAAAACTCATGTTCA	62.86	-0.10
		CCCGCCATAAAAACTCATGTTCAA	60.47	-0.10
		CCCCCATAAAAAACTCATGTTCAA \$	57.98	-0.10
	35	ccceataaaaaactcatcttcaad a	56.20	-0.10
2000		\		

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In one aspect of the present method, at least two parameters are determined wherein the parameters are poorly correlated with respect to one another. The reason for requiring that the different parameters chosen are poorly correlated with one another is that an additional parameter that is strongly correlated to the original parameter brings no additional information to the prediction process. The correlation to the original parameter is a strong indication that both parameters represent the same physical property of the system. Another way of stating this is that correlated parameters are linearly dependent on one another, while poorly correlated parameters are linearly independent of one another. In practice, the absolute value of the correlation coefficient between any two parameters should be less than 0.5, more preferably, less than 0.25, and, most preferably, as close to zero as possible.

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In one preferred approach instead of T_m, for each oligonucleotide/target nucleotide sequence duplex, the difference between the predicted duplex melting temperature corrected for salt concentration and the temperature of hybridization of each of the oligonucleotides with the target nucleotide sequence is determined.

In one aspect the present method comprises determining two parameters at least one of the parameters being the association free energy between a subsequence within each of the oligonucleotides and its complementary sequence on the target nucleotide sequence, or some similar, strongly correlated parameter. The object of this approach is to identify a particularly stable subsequence of the oligonucleotide that might be capable of acting as a nucleation site for the beginning of the heteroduplex formation between the oligonucleotide and the target nucleotide sequence. Such nucleation is believed to be the rate-limiting step for process of heteroduplex formation.

The subsequence within the oligonucleotide is from about 3 to 9 nucleotides in length, usually, 5 to 7 nucleotides in length. The subsequence is at least three nucleotides from the terminus of the oligonucleotide. For support-bound oligonucleotides the subsequence is at least three nucleotides from the free end of the oligonucleotide, i.e., the end that is not attached to the support. Generally, this free end is the 5' end of the oligonucleotide. When the oligonucleotide is attached to a support, the subsequence is at least three nucleotides from the end of the oligonucleotide that is bound to the surface of the support to which the oligonucleotide is attached. Generally, the 3' end of the oligonucleotide is bound to the support.

The predictive parameter can be, for example, either melting temperature or duplex free energy of the subsequence with the target nucleotide sequence. The subsequence with the maximum (melting temperature) or minimum (free energy) value of one of the above parameters is chosen as the representative subsequence for that oligonucleotide probe. For example, if the oligonucleotide is 20 nucleotides in length and a subsequence of 5 nucleotides is chosen, i.e., a 5-mer, then parameter values are calculated for all 5-mer subsequences of the oligonucleotide that do not include the 2 nucleotides at the free end of the oligonucleotide. Where 5' is the free end of the oligonucleotide with designated nucleotide number 1, the values are calculated for all 5-mer subsequences with

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starting nucleotides from position number 3 to position number 16. Thus, in this example, parameter values for 14 different subsequences are calculated. The subsequence with the maximum value for the parameter is then assigned as the stability subsequence for the oligonucleotide.

The inclusion of the above determination of a stability subsequence results in the following algorithm for determining the potential of an oligonucleotide to hybridize to a target nucleotide sequence. A predetermined number of unique oligonucleotides are identified within a nucleotide sequence that is hybridizable with said target nucleotide sequence. The oligonucleotides are chosen to sample the entire length of the nucleotide sequence. For each of the oligonucleotides, parameters that are independently predictive of the ability of each of said oligonucleotides to hybridize to said target nucleotide sequence are determined and evaluated. Two parameters that may be used are the thermodynamic parameters of T_m and ΔG_{MFOLD} . These parameters give rise to associated parameter filters. In one approach evaluation of the parameters involves establishing cut-off values as described above. Application of these cut-off values results in the identification of a subset of oligonucleotides for further scrutiny under the algorithm. In accordance with this embodiment of the present invention, there is included a stability subsequence limit in addition to the above. Cutoff values are determined either by means of objective optimization algorithms well known to the art or via graphical estimation methods; both approaches have been described previously in this document. In either case, the optimization of cutoff values involves comparison of predictions to known hybridization efficiency data sets. This process results in objective optimization as it looks at prediction versus experimental results and is otherwise referred to herein as "training the algorithm." The experimental data used to train the algorithm is referred to herein as "training data."

In the present approach filters are assigned to the T_m oligonucleotide probe data. The T_m of each oligonucleotide probe needs to be greater than or equal to the assigned filter (T_m probe limit) to be given a filter score of "1"; otherwise, the filter score is "0". In addition, one can also impose a second filter for this parameter; that is, that the T_m of the oligonucleotide probe also has to be less than a defined upper limit. Filters are also assigned to the ΔG_{MFOLD} data. The

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 ΔG_{MFOLD} of each oligonucleotide probe should be greater than or equal to the assigned filter (ΔG_{MFOLD} limit) to be given a filter score of "1"; otherwise, the filter score is "0". The filter scores are added. Furthermore, one can also impose a second filter for this parameter; that is, that the ΔG_{MFOLD} also has to be less than a defined upper limit. In accordance with the above discussion stability subsequences are identified. This leads to another filter. Accordingly, filters are assigned to the stability sequence data. The stability subsequence of each oligonucleotide probe needs to be greater than or equal to the assigned filter limit to be given a filter score of "1"; otherwise, the filter score is "0". In addition, one can also impose a second filter for this parameter; that is, that the stability subsequence also has to be less than a defined upper limit. In all cases, the filter values are determined by objective optimization (algorithmic or graphical) of the predictions of the present method versus training data, as described previously.

On the basis of the above filter sets a subset of oligonucleotides within said predetermined number of unique oligonucleotides is identified. Oligonucleotides in the subset are identified that are clustered along a region of the nucleotide sequence that is hybridizable to the target nucleotide sequence. The resulting number of oligonucleotide probe regions is examined. The above filters may then be loosened or tightened by changing the filter limits to obtain more or fewer clusters of oligonucleotides to match the goal, which is set by the needs of the investigator. For instance, a particular application might require that the investigator design 5 non-overlapping probes that efficiently hybridize to a given target sequence.

As mentioned above, the contigs may be selected on the basis of contig length. In another approach, the scores defined above may be summed for cluster size determination. To this end the probe score of the particular filter set (e.g., T_m probe limit, ΔG_{MFOLD} limit and stability sequence limit) is calculated for each oligonucleotide probe. The probe score is the sum of the filter scores. Thus, the probe score is 0 if no parameters pass their respective filters. The probe score is 1, 2 or 3 if one, two or three parameters, respectively, pass their filters for that oligonucleotide probe. This summing is continued for each parameter that is in the current filter set of the algorithm used. For a given algorithm a minimum probe score limit is set. In the current example this limit will be at least 1 and could be 2

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or 3 depending on the needs of the investigator, the number of probe clusters required and the results of objective optimizations of algorithm performance against training data. The probe score is compared to this probe score limit. If the probe score of oligonucleotide probe *i* is greater than or equal to the probe score limit, then oligonucleotide probe *i* is assigned a score passed value of 1. Next, a window is chosen for the evaluation of clustering (the "cluster window"). This will be the next filter applied. The cluster window ("w") smoothes the score passed values by summing the values in a window w nucleotides long, centered about position *i*. The resulting sum is called the cluster sum. Usually, the cluster window is an odd integer, usually 7 or 9 nucleotides. The cluster sum values are then filtered, by comparing to a user-set threshold, cluster filter. If cluster sum is greater than or equal to cluster filter, this filter is passed, and the probe is predicted to hybridize efficiently to its target.

This window summing procedure converts the score for the passed value for each oligonucleotide into a consensus metric for a set of *w* adjacent probes. A "consensus metric" is a measurement that distills a number of values into one consensus value. In this case, the consensus value is calculated by simply summing the individual values. The window summing procedure therefore evaluates a property similar to the contig length metric discussed above. However, the summed score has the advantage of allowing for a few probes within a cluster to have not passed their individual probe score limits. We have found that this allows more observed hybridization peaks to be predicted.

It may be desired in some circumstances to combine the results of multiple algorithm versions. We refer to this operation as "tiling". This may be explained more fully as follows. Tiling generally involves joining together the predicted oligonucleotide probe sets identified by multiple algorithm versions. In the context of the present invention, tiling multiple algorithm versions involves forming the union of multiple sets of predictions. These predictions may arise from different embodiments of the present invention. Alternatively, the different sets of predictions may arise from the same embodiment, but different filter sets. The different filter sets may additionally be restricted to different combinations of parameter values. For instance, one filter set might be used when the predicted

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duplex melting temperature T_m is greater than or equal to some value, while another might be used when T_m is below that value.

An example of the logical endpoint of tiling multiple filter sets across different regions of the possible combinations of predictive parameters and then forming the union of the resulting predictions is the contour plot shown in Fig. 3, with the associated rule that "the value of the normalized hybridization intensity associated with a particular combination of (T_m-T_{hyb}) and ΔG_{MFOLD} must be greater than or equal to some threshold value." In this case, the contour at the threshold value becomes the filter. This contour and its interior can be thought of as the union of many small rectangular regions ("tiles"), each of which is bracketed by low and high cutoff values for each of the parameters.

The predictions of different algorithm versions can also be combined by forming the intersection of two or more different predictions. The reliability of predictions within such intersection sets is enhanced because such sets are, by definition, insensitive to changes in the details of the predictive algorithm. Intersection is a useful method for reducing the number of predicted probes when a single algorithm version produces too many candidate probes for efficient experimental evaluation.

The most specific oligonucleotide probe set (i.e., the set least likely to include poor probes) will be the intersection set from multiple algorithms. Clusters that have overlapping oligonucleotide probes from multiple algorithms constitute the intersection set of oligonucleotide probes. The oligonucleotide probe that is in the center of an intersection cluster is chosen. This central oligonucleotide probe may have the highest probability of predicting a peak or, in other words, of binding well to the target nucleotide sequence. Oligonucleotide probes on either side of center, which are still within the intersection cluster, may also be selected. The distance of these "side" oligonucleotide probes from the center generally will be shorter or longer depending upon the length of the cluster.

The most sensitive set of oligonucleotide probes (i.e., the set most likely to include at least one good probe) is generally the union set from multiple algorithms. Clusters that are predicted by at least one type of algorithm constitute the union set of oligonucleotide probes. The oligonucleotide probe in the center of a union cluster is chosen. Oligonucleotide probes on either side of center, which

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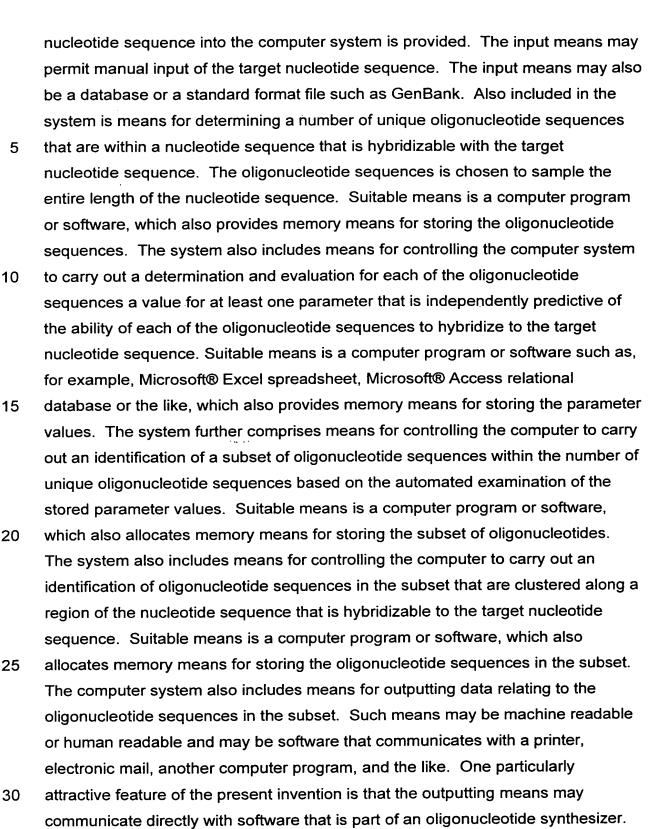
are still within the union cluster, usually are also chosen. The distance of these side probes from the center will be shorter or longer depending upon the length of the cluster. In summary, the combination of using the stability subsequence parameter, tiling multiple filter sets, and making union and intersection cluster sets of oligonucleotide probes exhibits very high sensitivity and specificity in predicting oligonucleotide probes that effectively hybridize to a target nucleotide sequence of interest.

Another aspect of the present invention is a computer based method for predicting the potential of an oligonucleotide to hybridize to a target nucleotide sequence. A predetermined number of unique oligonucleotides within a nucleotide sequence that is hybridizable with the target nucleotide sequence is identified under computer control. The oligonucleotides are chosen to sample the entire length of the nucleotide sequence. A value is determined and evaluated under computer control for each of the oligonucleotides for at least one parameter that is independently predictive of the ability of each of the oligonucleotides to hybridize to the target nucleotide sequence. The parameter values are stored. Based on the examination of the stored parameter values, a subset of oligonucleotides within the predetermined number of unique oligonucleotides is identified under computer control. Then, oligonucleotides in the subset that are clustered along a region of the nucleotide sequence that is hybridizable to the target nucleotide sequence are identified under computer control.

A computer program is utilized to carry out the above method steps. The computer program provides for input of a target-hybridizable or target-complementary nucleotide sequence, efficient algorithms for computation of oligonucleotide sequences and their associated predictive parameters, efficient, versatile mechanisms for filtering sets of oligonucleotide sequences based on parameter values, mechanisms for computation of the size of clusters of oligonucleotide sequences that pass multiple filters, and mechanisms for outputting the final predictions of the method of the present invention in a versatile, machine-readable or human-readable form.

Another aspect of the present invention is a computer system for conducting a method for predicting the potential of an oligonucleotide to hybridize to a target nucleotide sequence. An input means for introducing a target





In this way the results of the method of the present invention may be used directly

to provide instruction for the synthesis of the desired oligonucleotides.





Another advantage of the present invention is that it may be used to predict efficient hybridization oligonucleotides for each of multiple target sequences. Thus, very large arrays may be constructed and tested with minimal synthesis of oligonucleotides.

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EXAMPLES

The invention is demonstrated further by the following illustrative examples. Parts and percentages are by weight unless otherwise indicated. Temperatures are in degrees Centigrade (°C) unless otherwise specified. The following preparations and examples illustrate the invention but are not intended to limit its scope. All reagents used herein were from Amresco, Inc., Solon, Ohio (buffers), Pharmacia Biotech, Piscataway, N.J. (nucleoside triphosphates) or Promega, Madison, Wisconsin (RNA polymerases) unless indicated otherwise.

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Example 1

Synopsis: Data from labeled RNA target hybridizations to surface-bound DNA probes directed against 4 different gene sequences were compared to the predictions of the preferred version of the prediction algorithm illustrated by the flow chart in Fig. 2. The RNA targets were sequences derived from the human immunodeficiency virus protease-reverse transcriptase region (HIV PRT; sense-strand target polynucleotide), human glyceraldehyde-3-phosphate dehydrogenase gene (G3PDH; antisense-strand target polynucleotide), human tumor suppressor p53 gene (p53; antisense-strand target polynucleotide) and rabbit β-globin gene (β-globin; antisense-strand target polynucleotide). The GenBank accession numbers for the gene sequences, number of data points collected and temperature of hybridization have all been previously listed in Table 2.

30 <u>Materials and Methods</u>: Three different experimental systems and two different labeling schemes were used to collect data.

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The sequence and hybridization data for β -globin were taken from the literature (see Milner *et al.*, (1997), *supra*; in this experiment, ³²P-radiolabeled RNA target was used.

The hybridization data for HIV PRT were obtained using an Affymetrix GeneChip™ HIV PRT-sense probe array (i.e. sense strand target polynucleotide) (GeneChip™ HIV PRT 440s, Affymetrix Corporation, Santa Clara, California) as specified by the manufacturer, except that the fluorescein-labeled RNA target was not fragmented prior to hybridization and that hybridization was performed for 24 hours. The concentration of fluorescein-labeled RNA used was 26.3 nM; label density was approximately 18 fluoresceinated uridyl nucleotides per 1 kilobase (kb) RNA transcript. The raw data were collected by scanning the array with a GeneChip™ Scanner 50 (Affymetrix Corporation, Santa Clara, California), as specified by the manufacturer. The raw data were reduced to a feature-averaged (".CEL") file, using the GeneChip™ software supplied with the scanner. Finally, a table of hybridization intensities for perfect-complement 20-mer probes was constructed using the ASCII feature map file supplied with the GeneChip™ software to connect probe sequences to measured hybridization intensities. The resulting data set contained data for every overlapping 20-mer probe to the target sequence.

The data for G3PDH and p53 were measured using 93-feature arrays constructed using commercially available streptavidin-coated microtiter plates (Pierce Chemical Company, Rockford, IL). Every tenth possible 25-mer probe complementary to each target was synthesized and 3'-biotinylated by a contract synthesis vendor (Operon, Inc., Alameda, CA). The 3'-linked biotin was used to anchor individual probes to microtiter wells, via the well known, strong affinity of streptavidin for biotin. Biotinylated DNA probes were resuspended to a concentration of 10 μM in hybridization buffer (5x sodium chloride-sodium phosphate-disodium ethylenediaminetetraacetate (SSPE), 0.05% Triton X-100, filter-sterilized; 1x SSPE is 150 mM sodium chloride, 10 mM sodium phosphate, 1 mM disodium ethylenediaminetetraacetate (EDTA), pH 7.4). Individual probes were diluted 1:10 in hybridization buffer into specified wells (100 μl total volume per well) of a streptavidin-coated microtiter plate; probes were allowed to bind to the covered plates overnight at 35°C. The other 3 wells of the 96-well microtiter

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plate were probe-less controls. The coated plates were washed with 3 x 200 µl of wash buffer (6x SSPE, 0.005% Triton X-100, filter-sterilized). Fluorescein-labeled RNA (100 µl of a 10 nM solution in hybridization buffer) was added to each well. The plates were covered and hybridized at 35°C for 20-24 hours. The hybridized plates were washed with 3 x 200 µl of wash buffer. Label was then released in each well by adding 100 µl of 20 µg/ml RNAase I (Sigma Chemical Company, St. Louis, MO) in Tris-EDTA (TE) (10 mM Tris(hydroxymethyl)aminomethane (Tris), 1 mM EDTA, pH 8.0, sterile) and incubating at 35°C for at least 30 minutes. The fluorescence released from the surface of each well was quantitated with a PerSeptive Biosystems Cytofluor II microtiter plate fluorimeter (PerSeptive Biosystems, Inc., Framingham, MA) using the manufacturer's recommended excitation and emission filter sets for fluorescein. Each plate hybridization was performed in quadruplicate, and the data for each probe were averaged to obtain the hybridization intensity.

Labeled RNA targets specific for G3PDH and p53 were produced via T7 RNA polymerase transcription of DNA templates in the presence of fluorescein-UTP (Boehringer Mannheim Corporation, Indianapolis, IN), using the same method as that outlined by Affymetrix for their GeneChip™ HIV PRT sense probe array. The DNA template for G3PDH was purchased from a commercial source (Clontech, Inc., Palo Alto, CA). The DNA template for p53 was obtained by subcloning a PCR fragment from an ATCC-derived reference clone (No. 57254) of human p53 into the commercially-available PCR cloning vector pCR2.1-TOPO (Invitrogen, Inc., Carlsbad, CA), then linearizing the plasmid at the end of the polycloning site opposite the vector-derived T7 promoter.

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Probe predictions were performed using a software application (referred to as "p5") that was built atop Microsoft's Access relational database application, using added Visual Basic modules, the TrueDB Grid Pro 5.0 (Apex Software Corporation, Pittsburgh, PA) enhancement to Visual Basic, and a version of the FORTRAN application MFOLD, modified to run in a Windows NT 4.0 environment, as an ActiveX control. The Visual Basic source code for the p5 software application is found in the Microfiche appendix to this specification. The DNA target sequence complements that were input into p5 for division into potential oligonucleotide probe sequences are listed below:





Parent Sequence Accession No.: K03256

Locus: BUNGLOB.DNA (portion of rabbit β -globin)

Length: 122

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1 TTCTTCCACA TTCACCTTGC CCCACAGGGC AGTGACCGCA GACTTCTCCT CACTGGACAG 61 ATGCACCATT CTGTCTGTTT TGGGGGATTG CAAGTAAACA CAGTTGTGTC AAAAGCAAGT

121 GT SEQ ID NO:36

10 Parent Sequence Accession No.: M15654

Locus: HIV_PRTA.S (HIV PRT antisense; parses into probes specific for

sense-strand target)

Length: 1040

1 TGTACTGTCC ATTTATCAGG ATGGAGTTCA TAACCCATCC AAAGGAATGG AGGTTCTTTC 61 TGATGTTTTT TGTCTGGTGT GGTAAGTCCC CACCTCAACA GATGTTGTCT CAGCTCCTCT 15 121 ATTTTTGTTC TATGCTGCCC TATTTCTAAG TCAGATCCTA CATACAAATC ATCCATGTAT 181 TGATAGATAA CTATGTCTGG ATTTTGTTTT TTAAAAAGGCT CTAAGATTTT TGTCATGCTA 241 CTTTGGAATA TTGCTGGTGA TCCTTTCCAT CCCTGTGGAA GCACATTGTA CTGATATCTA 301 ATCCCTGGTG TCTCATTGTT TATACTAGGT ATGGTAAATG CAGTATACTT CCTGAAGTCT 361 TCATCTAAGG GAACTGAAAA ATATGCATCA CCCACATCCA GTACTGTTAC TGATTTTTTC 20 421 TTTTTTAACC CTGCGGGATG TGGTATTCCT AATTGAACTT CCCAGAAGTC TTGAGTTCTC 481 TTATTAAGTT CTCTGAAATC TACTAATTTT CTCCATTTAG TACTGTCTTT TTTCTTTATG 541 GCAAATACTG GAGTATTGTA TGGATTCTCA GGCCCAATTT TTGAAATTTT CCCTTCCTTT 601 TCCATTTCTG TACAAATTTC TACTAATGCT TTTATTTTTT CTTCTGTCAA TGGCCATTGT 661 TTAACTTTTG GGCCATCCAT TCCTGGCTTT AATTTTACTG GTACAGTCTC AATAGGGCTA 25 721 ATGGGAAAAT TTAAAGTGCA ACCAATCTGA GTCAACAGAT TTCTTCCAAT TATGTTGACA 781 GGTGTAGGTC CTACTAATAC TGTACCTATA GCTTTATGTC CACAGATTTC TATGAGTATC 841 TGATCATACT GTCTTACTTT GATAAAACCT CCAATTCCCC CTATCATTTT TGGTTTCCAT 901 CTTCCTGGCA AACTCATTTC TTCTAATACT GTATCATCTG CTCCTGTATC TAATAGAGCT 961 TCCTTTAGTT GCCCCCCTAT CTTTATTGTG ACGAGGGGTC GTTGCCAAAG AGTGATCTGA 30

Parent Sequence Accession No.: X01677

1021 GGGAAGTTAA AGGATACAGT SEQ ID NO:37

Locus: G3PDH (Clontech G3PDH template - parses into probes specific for

antisense-strand target)

Length: 999

			CECTT CCCTE	mmccmccm n m	TCCCCCCCTC	GTCACCAGGG	$CTCCTTTT\Delta\Delta$
	1	GAAGGTCGGA	GTCAACGGAT	TIGGICGIAI	IGGGCGCCIG	GICACCAGGG	CIGCIIII
40	61	CTCTGGTAAA				ATTGACCTCA	
	121	TTACATGTTC	CAATATGATT	CCACCCATGG	CAAATTCCAT	GGCACCGTCA	AGGCTGAGAA
	181	CGGGAAGCTT	GTCATCAATG	GAAATCCCAT	CACCATCTTC	CAGGAGCGAG	ATCCCTCCAA
	241	AATCAAGTGG	GGCGATGCTG	GCGCTGAGTA	CGTCGTGGAG	TCCACTGGCG	TCTTCACCAC
	301	CATGGAGAAG	GCTGGGGCTC	ATTTGCAGGG	GGGAGCCAAA	AGGGTCATCA	TCTCTGCCCC
45	361	CTCTGCTGAT	GCCCCCATGT	TCGTCATGGG	TGTGAACCAT	GAGAAGTATG	ACAACAGCCT
	421	CAAGATCATC	AGCAATGCCT	CCTGCACCAC	CAACTGCTTA	GCACCCCTGG	CCAAGGTCAT
	481	CCATGACAAC	TTTGGTATCG	TGGAAGGACT	CATGACCACA	GTCCATGCCA	TCACTGCCAC
	541	CCAGAAGACT	GTGGATGGCC	CCTCCGGGAA	ACTGTGGCGT	GATGGCCGCG	GGGCTCTCCA
	601	GAACATCATC	CCTGCCTCTA	CTGGCGCTGC	CAAGGCTGTG	GGCAAGGTCA	TCCCTGAGCT
50	661	AGACGGGAAG	CTCACTGGCA	TGGCCTTCCG	TGTCCCCACT	GCCAACGTGT	CAGTGGTGGA
	721	CCTGACCTGC	CGTCTAGAAA	AACCTGCCAA	ATATGATGAC	ATCAAGAAGG	TGGTGAAGCA
	781	GGCGTCGGAG	GGCCCCTCA	AAGGCATCCT	GGGCTACACT	GAGCACCAGG	TGGTCTCCTC
	841			ACTCCTCCAC			TTGCCCTCAA
	901	CGACCACTTT		TTTCCTGGTA			GCAACAGGGT
55	961	GGTGGACCTC	ATGGCCCACA	TGCTATAGTG	AGTCGTATT	SEQ ID NO:38	3

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Parent Sequence Accession No.: X54156

Locus: HSP53PCRa (p53 template - parses into probes specific for

antisense-strand target)

Length: 1049

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	1				GACCGGCGCA		
	61				CCAGGGAGCA		
	121				AAACCACTGG		
	181				TTCCGAGAGC		
10	241				GGGAGCAGGG		
	301	TCCAAAAAGG	GTCAGTCTAC	CTCCCGCCAT	AAAAAACTCA		
	361	GACTCAGACT				AGCCTCCCTC	
	421	CCCTCCCCTG			TTGAACCCTT		
	481	AGAAGCACCC	AGGACTTCCA	TTTGCTTTGT	CCCGGGGCTC	CACTGAACAA	GTTGGCCTGC
15	541	ACTGGTGTTT					
	601				TAAGAAATGT		
	661				GGCCCACTTC		CCAGGGAAGC
	721	TGTCCCTCAT	GTTGAATTTT	CTCTAACTTC	AAGGCCCATA		GCTGGCATTT
	781	GCACCTACCT	CACAGAGTGC	ATTGTGAGGG	TTAATGAAAT	AATGTACATC	
20	841	ACCACCTTTT	ATTACATGGG	GTCTAAAACT	TGACCCCCTT		GTTCCCTCTC
	901	CCTCTCCCTG	TTGGCTGGTG	GGTTGGTAGT		GGGCAGCTGG	
	961	GGAGTTGTCA			ACCCTGTCTG		GGTCGACCTT
	1021	AGTACCTAAA	AGGAAATCTC	ACCCCATCC	SEQ ID NO:39	9	

The sequences indicated above, which are complements of the target sequences, were divided into overlapping oligonucleotide sequences with one nucleotide between starting positions. The oligonucleotide sequence lengths were 17 (rabbit β -globin), 20 (HIV PRT) or 25 (G3PDH; p53). The oligonucleotide sequence lengths were dictated by the probe lengths used in the experiments to which the predictions were compared. The RNA target concentrations used to calculate predicted RNA/DNA duplex melting temperatures were 100 pM (rabbit β -globin), 26.3 nM (HIV PRT) and 10 nM (G3PDH; p53). These were also dictated by experimental conditions for the comparison data. The cut-off filter used for the predicted free energy of the most stable probe sequence intramolecular structure,

35 ΔG_{MFOLD} , was

$$\Delta G_{MFOLD} \ge -0.4 \frac{\text{kcal}}{\text{mole}}$$
.

The filter condition used for the predicted RNA/DNA duplex melting temperature was

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$$25^{\circ}C \le T_m + 16.6 \log([Na^+]) - T_{hyb} \le 50^{\circ}C$$
,

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where T_m is the target concentration-dependent value of the predicted RNA/DNA duplex melting temperature before correction for salt concentration, the term "16.6 $\log([\mathrm{Na^+}])$ " corrects the melting temperature for salt effects, and T_{hyb} is the hybridization temperature. The values of the salt correction term and T_{hyb} have already been listed in Table 2. For convenient use within p5, the above condition was algebraically rearranged into the equivalent form

$$25^{\circ}C - 16.6 \log([Na^{+}]) + T_{hyh} \leq T_{m} \leq 50^{\circ}C - 16.6 \log([Na^{+}]) + T_{hyh}.$$

Clusters were ranked according to the number of contiguous oligonucleotide sequences that passed through the filter set ("contig" length).

Results: The detailed analysis results for rabbit β -globin are presented in Table 3; a graphical summary of the results is shown in Fig. 4. In Table 3, values of T_m and ΔG_{MFOLD} that were excluded by the filter set are shown with a line through them, and table entries for contig length are shown in gray when the oligonucleotide sequence in question was not in a contig. The top 20% of the observed hybridization intensities are shown underlined.





Table 3

Position	Oligonucleotide Sequence	SEQ ID NO:	T _m (°C)	ΔG _{MFOLD} (kcal/mole)	Contig Length	Hybridization Intensity (Milner et al., 1997)
1	TTCTTCCACATTCACCT	40	53.62	5.00		100
2	TCTTCCACATTCACCTT	41	53.62	5.00		130
3	CTTCCACATTCACCTTG	42	52.19	0.90		130
4	TTCCACATTCACCTTGC	43	54.50	0.50		200
5	TCCACATTCACCTTGCC	44	58.46	0.50	7	120
6	CCACATTCACCTTGCCC	45	61.10	0.50	7	180
7	CACATTCACCTTGCCCC	46	61.10	0.50	7	230
8	ACATTCACCTTGCCCCA	47	61.10	0.50	7	220
9	CATTCACCTTGCCCCAC	48	61.10	0.90	7	320
10	ATTCACCTTGCCCCACA	49	61.10	0.70	. 7	<u>310</u>
11	TTCACCTTGCCCCACAG	50	61.33	0.50	7	320
12	TCACCTTGCCCCACAGG	51	63.70	-0.60		<u>390</u>
13	CACCTTGCCCCACAGGG	52	64.85	-1.60		410
14	ACCTTGCCCCACAGGGC	53	68.01	-4.10		240
15	CCTTGCCCCACAGGGCA	54	68.63	-5.40		50
16	CTTGCCCCACAGGGCAG	55	64.95	1 1		20
17	TTGCCCCACAGGGCAGT	56	66.31	1		20
18	TGCCCCACAGGGCAGTG	57	65.79	-5.40		20
19	GCCCCACAGGGCAGTGA	58	67.37	-4.10		20
20	CCCCACAGGGCAGTGAC	59	63.42	-1.60		40
21	CCCACAGGGCAGTGACC	60	63.42			20
22	CCACAGGGCAGTGACCG	61	59.85			20
23	CACAGGGCAGTGACCGC	62	60.14	1		20
24	ACAGGGCAGTGACCGCA	63	60.14			20
25	CAGGGCAGTGACCGCAG	64	59.76			30
26	AGGGCAGTGACCGCAGA	65	59.83			20
27	GGGCAGTGACCGCAGAC	66	60.22			30
28	GGCAGTGACCGCAGACT	67	59.53			30
29	GCAGTGACCGCAGACTT	68	57.06			30
30	CAGTGACCGCAGACTTC	69	53.90			40
31	AGTGACCGCAGACTTCT	70	54.71			40
32	GTGACCGCAGACTTCTC	71	55.99	i	7	
33	TGACCGCAGACTTCTCC	72	57.01			
34	GACCGCAGACTTCTCCT	73	59.22			
35	ACCGCAGACTTCTCCTC	74	59.28			
36	CCGCAGACTTCTCCTCA	75	60.07			
37	CGCAGACTTCTCCTCAC	76	56.34		l	
38	GCAGACTTCTCCTCACT	77	57.79	1	<u> </u>	
39	CAGACTTCTCCTCACTG	78	52.93			240
40	AGACTTCTCCTCACTGG	79	54.4	0.00		340





Table 3

Position	Oligonucleotide Sequence	SEQ ID NO:	T _m (°C)	ΔG _{MFOLD} (kcal/mole)	Contig Length	Hybridization Intensity (Milner et al., 1997)
41	GACTTCTCCTCACTGGA	80	55.77	-1.40		340
42	ACTTCTCCTCACTGGAC	81	54.85	-1.60		240
43	CTTCTCCTCACTGGACA	82	55.75	-1.60		240
44	TTCTCCTCACTGGACAG	83	53.66	-1.60		120
45	TCTCCTCACTGGACAGA	84	54.82	-1.60		100
46	CTCCTCACTGGACAGAT	85	53.36	-1.60		110
47	TCCTCACTGGACAGATG	86	51.10	-1.40		80
48	CCTCACTGGACAGATGC	87	54.25	0.00		240
49	CTCACTGGACAGATGCA	88	51.26	0.20		90
50	TCACTGGACAGATGCAC	89	49.63	0.20		30
51	CACTGGACAGATGCACC	90	52.74	0.50	•	100
52	ACTGGACAGATGCACCA	91	52.74	-0.50		80
53	CTGGACAGATGCACCAT	92	52.18	-1.00		90
54	TGGACAGATGCACCATT	93	50.39	-0.80		80
55	GGACAGATGCACCATTC	94	51.75	0.30		180
56	GACAGATGCACCATTCT	95	51.05	-0.10		220
57	ACAGATGCACCATTCTG	96	49.56	-1.80		120
58	CAGATGCACCATTCTGT	97	52.19	-2.10		120
59	AGATGCACCATTCTGTC	98	52.06	-0.10		250
60	GATGCACCATTCTGTCT	99	54.18	0.30		520
61	ATGCACCATTCTGTCTG	100	52.60	0.40		<u>980</u>
62	TGCACCATTCTGTCTGT	101	56.05	0.20	2	780
63	GCACCATTCTGTCTGTT	102	56.52	0.20	2	<u>810</u>
64	CACCATTCTGTCTGTTT	103	52.06	IL		220
65	ACCATTCTGTCTGTTTT	104	50.83	0.20		120
66	CCATTCTGTCTGTTTTG	105	50.18	0.20		120
67	CATTCTGTCTGTTTTGG	106	48.42			160
68	ATTCTGTCTGTTTTGGG	107	49.91	1.70		<u>310</u>
69	TTCTGTCTGTTTTGGGG	108	53.10			<u>250</u>
70	TCTGTCTGTTTTGGGGG	109	55.90	l l	2	80
71	CTGTCTGTTTTGGGGGA	110	55.91	1.40	2	30
72	TGTCTGTTTTGGGGGAT	111	53.55	l		50
73	GTCTGTTTTGGGGGATT	112	54.00	1		10
74	TCTGTTTTGGGGGATTG	113	50.50	<u> </u>		10
75	CTGTTTTGGGGGATTGC	114	53.77			10
76	TGTTTTGGGGGATTGCA	115	53.04			10
77	GTTTTGGGGGATTGCAA	116	51.01	0.00		5
78	TTTTGGGGGATTGCAAG	117	47.99	-0.20		5
79	TTTGGGGGATTGCAAGT	118	50.80	-0.20		5
80	TTGGGGGATTGCAAGTA	119	49.80	0.00		5

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Table 3

Position	Oligonucleotide Sequence	SEQ ID NO:	T _m (°C)	ΔG _{MFOLD} (kcal/mole)	Contig Length	Hybridization Intensity (Milner <i>et al.</i> , 1997)
81	TGGGGGATTGCAAGTAA	120	47.55	1.20		5
82	GGGGGATTGCAAGTAAA	121	45.76	1.40		5
83	GGGGATTGCAAGTAAAC	122	43.54	1.40		5
84	GGGATTGCAAGTAAACA	123	42.32	1.30		5
85	GGATTGCAAGTAAACAC	124	40.11	0.90		5
86	GATTGCAAGTAAACACA	125	38.94	0.50		5
87	ATTGCAAGTAAACACAG	126	37.61	0.50		5
88	TTGCAAGTAAACACAGT	127	40.35	0.50		5
89	TGCAAGTAAACACAGTT	128	40.35	0.30		5
90	GCAAGTAAACACAGTTG	129	40.35	0.10		10
91	CAAGTAAACACAGTTGT	130	38.98	-0.30	-	5
92	AAGTAAACACAGTTGTG	131	37.40	-0.90		5
93	AGTAAACACAGTTGTGT	132	42.02	-2.30	•	5
94	GTAAACACAGTTGTGTC	133	43.15	-2.50		5
95	TAAACACAGTTGTGTCA	134	41.73	-2.50		5
96	AAACACAGTTGTGTCAA	135	40.67	-2.50		5
97	AACACAGTTGTGTCAAA	136	40.67	-2.50		5
98	ACACAGTTGTGTCAAAA	137	40.67	l		10
99	CACAGTTGTGTCAAAAG	138	40.20	-1.20		15
100	ACAGTTGTGTCAAAAGC	139	42.93	-0.50		30
101	CAGTTGTGTCAAAAGCA	140	43.99	0.20		25
102	AGTTGTGTCAAAAGCAA	141	40.67	-0.10		25
103	GTTGTGTCAAAAGCAAG	142	40.67	1		20
104	TTGTGTCAAAAGCAAGT	143	40.67			120
105	TGTGTCAAAAGCAAGTG	144	40.40	0.50		20

In Fig. 4, the hybridization intensity observed experimentally is plotted as a function of oligonucleotide starting position in the target-complementary sequence that was input into p5. The identified contigs are plotted as horizontal bars, with the contig rank (by length) shown in parentheses next to each bar. It is clear from Table 3 and Fig. 4 that the prediction algorithm identified contigs that overlap all of the "top 20%" hybridization intensity peaks observed. Iterative experimental improvement of these predictions would converge on each of the observed intensity maxima in 3-4 iterations.

Prediction worksheets for HIV PRT, G3PDH and p53 were prepared in a manner similar to that for rabbit β -globin as shown in Table 3, except that the probes were longer as indicated above and that approximately 1,000 probes were

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analyzed for each of these genes. The results of these analyses are shown in Fig. 5 (HIV PRT), Fig. 6 (G3PDH) and Fig. 7 (p53). In Fig. 5, data are plotted for all possible 20-mer oligonucleotide probes. In Figs. 6 and 7, data were available for only every 10th 25-mer probe, and the actual data points are plotted as open diamonds.

It is clear from Figs. 5-7 that the hybridization efficiency prediction algorithm of the present invention performed well in the task of identifying regions with observed high hybridization intensity. In each case, the 4 longest contigs point to good-toexcellent regions for experimental investigation. It should be noted that the contigs usually bracket observed intensity peaks; experimental iterative refinement would therefore be expected to converge in 2-3 iterations. By this is meant that certain oligonucleotides from the identified contigs are prepared and subjected to evaluation in actual hybridization experiments. Based on the results of such experiments, the observed signal is evaluated to determine whether the oligonucleotides are hybridizing to the left of, the right of, or on the center of a peak with respect to the graphed data. The next iteration is carried out to experimentally evaluate the hybridization efficiency of probes that are inferred to lie closer to the peak of hybridization efficiency, based on the data from the previous iteration. Iteration is continued until the signal level is deemed acceptable by the user, or the local hybridization efficiency maximum is reached (i.e. the best probe in the cluster identified by the method of the current invention has been experimentally identified). A detailed illustration of this process is shown in Example 3.

It should be noted that clusters of predictions that overlap the maxima of observed peaks of hybridization efficiency will often yield user-acceptable probes on the first iteration. Thus, the method of the present invention is much more efficient than current methods in which every potential probe is synthesized. For instance, in the HIV PRT example shown in Fig. 5, at least 3 good probes would be identified after synthesis of ~10 test probes (i.e. statistical sampling of the 3 longest contigs). This is much more efficient than the ~1,000 probes represented by the data in Fig. 5.

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Example 2

Synopsis: Data from a labeled RNA target hybridization to an Affymetrix GeneChip™ HIV PRT-sense probe array (GeneChip™ HIV PRT 440s, Affymetrix Corporation, Santa Clara, CA) were compared to the predictions of the window-averaged composite dimensionless score version of the method of the present invention.

Materials and Methods: Data were obtained as described for the Affymetrix GeneChip™ HIV PRT-sense probe array (GeneChip™ HIV PRT 440s, Affymetrix Corporation, Santa Clara, California) in Example 1. The DNA sequence (SEQ ID NO: 37) complementary to the fluorescein-labeled RNA target was divided into overlapping 20-mer oligonucleotide sequences spaced one nucleotide apart, using the prototype application p5; p5 was also used to calculate the predicted values of the RNA/DNA heteroduplex melting temperature (T_m) and the free energy of the most stable predicted probe intramolecular structure, ΔG_{MFOLD} , as described in Example 1. The probe sequences and parameter values were then transferred to a Microsoft Excel spreadsheet, which was used to complete the predictions of efficient and inefficient probes. The weight was obtained by optimizing the performance of the algorithm with the data of Milner et al., supra, as the training data using the Microsoft® Excel® spreadsheet software. The composite score was calculated using a weight of 0.62 for the dimensionless T_m score and a weight of 0.38 for the ΔG_{MFOLD} dimensionless score. The windowedaveraging was performed using a window width of 7 and Microsoft® Excel® spreadsheet software. Finally, the oligonucleotide sequences having the top 10% of the window-averaged composite dimensionless scores were predicted to be efficient probes, while the oligonucleotide sequences having the bottom 10% of the window-averaged composite dimensionless scores were predicted to be inefficient probes.

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Results: The calculated parameters and scores are shown in Table 4; the algorithm predictions are also shown diagrammatically in Figure 8. In Table 4, window-averaged composite score values that were in the top 10% of the





distribution of values are shown in bold type, values that were in the bottom 10% are shown in italics, and all other values are shown with a line through them. It is clear from both Table 4 and Figure 8 that the window-averaged composite dimensionless score embodiment of the current invention correctly predicted both efficient and inefficient hybridization probes for HIV PRT sense-strand RNA. As in Example 1, statistical sampling of contiguous stretches of predicted "good" probes would lead to convergence of the design process to the best probes in each region in 2-4 design iterations.

Table 4

																										,
HIV PRT GeneChip™ Data	1152.2	1040.7	291.9	221.8	148.3	84.6	128.7	94.6	157.5	316.9	360.2	403.8	382.5	324.4	320.5	238.9	202.3	113.6	2.79	143.3	484.6	857.6	991.4	0.706	887.9	1015.3
Window- Averaged Composite Score				-0.168	-0.284	-0.308	0.252	0.242	-0.236	-0.227	-0.194	-0.105	0.014	400.0		-0.101		0.120		001'0'	601'0	•	0.053		0,137	0.053
Composite Score	0.269	-0.125	-0.246	-0.059	-0.117	-0.418	-0.484	-0.519	-0.317	0.146	0.014	-0.073	-0.354	-0.252	0.104	0.315	0.216	-0.202	-0.533	-0.746	0.007	0.249	0.307	0.155	0.155	0.246
∆GMFOLD Score	-0.199	-0.460	-0.895	-0.895	-0.895	-1.764	-1.938	-1.764	-0.634	0.583	0.236	0.236	0.062	0.062	0.062	0.062	-0.199	-1.243		-2.982	-1.851	-1.504	-0.286	0.062	0.062	0.410
Score	0.557	0.080	0.152	0.453	0.360	0.408	0.408	0.245	-0.122	-0.122	-0.122	-0.262	-0.609	-0.444	0.129	0.470	0.470	0.436		0.624	1.146	1.324	0.670	0.213	0.213	0.145
∆GMFOLD (kcal/mole @ 35 °C)	-0.10	-0.40	-0.90	06.0-	-0.90	-1.90	-2.10	-1.90	-0.60	08.0	0.40	0.40	0.20	0.20	0.20	0.20	-0.10	-1.30	-2.00	-3.30	-2.00	-1.60	-0.20	0.20	0.20	09.0
RNA/DNA Tm (°C)	64.16	60.91	61.41	63.46	62.82	63.15	63.15	62.03	59.53	59.53	59.53	58.58	56.21	57.34	61.25	63.57	63.57	63.34	62.24	64.62	68.18	69.39	64.93	61.82	61.82	61.36
SEQ ID NO:	145	146	147	148	149	150	151	152	153	154	155	156	157	158	159	160	161	162	163	164	165	166	167	168	169	170
DNA Probe Sequence	1 GTACTGTCCATTTATCAGGA	2 TACTGTCCATTTATCAGGAT	3 ACTGTCCATTTATCAGGATG	4 CTGTCCATTTATCAGGATGG	5 TGTCCATTTATCAGGATGGA	6 GTCCATTTATCAGGATGGAG	7 TCCATTTATCAGGATGGAGT	8 CCATTTATCAGGATGGAGTT	9 CATTTATCAGGATGGAGTTC	10 ATTTATCAGGATGGAGTTCA	11 TTTATCAGGATGGAGTTCAT	12 TTATCAGGATGGAGTTCATA	13 TATCAGGATGGAGTTCATAA	14 ATCAGGATGGAGTTCATAAC	15 TCAGGATGGAGTTCATAACC	16 CAGGATGGAGTTCATAACCC	17 AGGATGGAGTTCATAACCCA	18 GGATGGAGTTCATAACCCAT	19 GATGGAGTTCATAACCCATC	20 ATGGAGTTCATAACCCATCC	21 TGGAGTTCATAACCCATCCC	22 GGAGTTCATAACCCATCCCA	23 GAGTTCATAACCCATCCCAA	24 AGTTCATAACCCATCCCAAA	25 GTTCATAACCCATCCCAAAG	26 TTCATAACCCATCCCAAAGG
p5 Probe Position	1	2	3	4	5	9	7	8	6	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26



ԴRT hip™	<u> </u>	7 076	210.7	170.7	0.00 0.00	0.1.0	42 9	45.0	45.3	47.9	49.2	74.2	125.5	183.3	2614	518.3	716.5	1056.0	1084 3	1241.1	1278.8	1616.0	1677.5	1963.1	2126 1	2143.3	3540 B	0.0
HIV PRT GeneChip™	Data																								2	2	C.	,
Window- Averaged	Score	0000	0.124	0.204	0300	0.412	0.488	-0.542	-0.579	-0.587	0.580	0.585	0.572	-0.485	0.380	-0.277	0.206	0.164	-0.025	0.119	0.217	0.258	0.270	0000	0.304	0.234	0 180	201.0
Composite Score		0 092	-0.243	-0.340	-0.345	-0.435	-0.401	-0.494	-0.623	-0.778	-0.721	-0.600	-0.490	-0.356	-0.530	-0.529	-0.170	0.015	0.119	0.008	-0.057	0.440	0.476	0.522	0.300	0.202	0.215	2
∆GMFOLD Score		-0.199	-0.373	-0 373	-0.373	-1.243	-1.851	-2.025	-2.112	-2.373	-2.025	-2.025	-1.851	-0.721	-0.373	-0.373	-0.373	-0.373	-0.373	-0.025	0.149	0.149	0.149	0.149	0.149	0.149	0.149	
Score		0.270	-0.163	-0.320	-0.328	0.061	0.487	0.445	0.290	0.199	0.079	0.274	0.344	-0.132	-0.627	-0.625	-0.046	0.253	0.420	0.028	-0.183	0.618	0.677	0.751	0.392	0.235	0.256	
ΔG _{MFOLD} (kcal/mole	3 3 3	-0.10	-0.30	-0.30	-0.30	-1.30	-2.00	-2.20	-2.30	-2.60	-2.20	-2.20	-2.00	-0.70	-0.30	-0.30	-0.30	-0.30	-0.30	0.10	0.30	0.30	0.30	0.30	0.30	0.30	0.30	
Tm (°C)		62.21	59.26	58.19	58.13	82.09	63.69	63.40	62.34	61.72	06.09	62.24	62.71	59.47	56.10	56.11	60.05	65.09	63.23	99.09	59.12	64.58	64.98	62.49	63.04	61.97	62.11	
NO:		171	172	173	174	175	176	177	178	179	180	181	182	183	184	185	186	187	188	189	190	191	192	193	194	195	196	
egienhes agoil Cur		27 TCATAACCCATCCCAAAGGA	28 CATAACCCATCCCAAAGGAA	29 ATAACCCATCCCAAAGGAAT	30 TAACCCATCCCAAAGGAATG	31 AACCCATCCCAAAGGAATGG	32 ACCCATCCCAAAGGAATGGA	33 CCCATCCCAAAGGAATGGAG	34 CCATCCCAAAGGAATGGAGG	35 CATCCCAAAGGAATGGAGGT	36 ATCCCAAAGGAATGGAGGTT	37 TCCCAAAGGAATGGAGGTTC	38 CCCAAAGGAATGGAGGTTCT	39 CCAAAGGAATGGAGGTTCTT	40 CAAAGGAATGGAGGTTCTTT	41 AAAGGAATGGAGGTTCTTTC	42 AAGGAATGGAGGTTCTTTCT	43 AGGAATGGAGGTTCTTTCTG	44 GGAATGGAGGTTCTTTCTGA	45 GAATGGAGGTTCTTTCTGAT	46 AATGGAGGTTCTTTCTGATG	47 ATGGAGGTTCTTTCTGATGT	48 TGGAGGTTCTTTCTGATGTT	49 GGAGGTTCTTTCTGATGTTT	50 GAGGTTCTTTCTGATGTTTT	51 AGGTTCTTTCTGATGTTTTT	52 GGTTCTTTCTGATGTTTTTT	できる の中ではなる 多くせん 中中での中でしている
Position		27	28	29	30	31	32	33 (34 (35 (36 7	37 [) 88) 66 7	40 0	411	42 1	437	44	45	46 7	47 1	48 1	49	20 0	5114	52 G	(CL

TC CT CC CO TAKEN BACE CO -72-

Table 4

	NO:	RNA/DNA T _m (°C)	∆G _{MFOLD} (kcal/mole @ 35 °C)	Score	∆GMFOLD Score	Composite Score	Window- Averaged Composite	HIV PRT GeneChip [™] Data
54 TTCTTTCTGATGTTTTTTGT	198	59.21	0:0	-0.170	0 149	-0.049	Score 0.151	1364 3
55 TCTTTCTGATGTTTTTGTC	199	60.35			0.323	0.121	0.183	1788 4
56 CTTTCTGATGTTTTTTGTCT	200	96.09			0.931	0.407	0.253	2670.9
57 TTTCTGATGTTTTTTGTCTG	201	58.76	1.20	-0.235	0.931	0.208		3336.2
58 TTCTGATGTTTTTTGTCTGG	202	61.17	1.20	0.118	0.931	0.427	0.440	6683.6
59 TCTGATGTTTTTTGTCTGGT	203	64.20	l	0.562	0.931	0.702	0.537	10227.0
60 CTGATGTTTTTTGTCTGGTG	204	62.51	1.20	0.315	0.931	0.549	0.625	10965.0
61 TGATGTTTTTTGTCTGGTGT	205	63.80	1.20	0.504	0.931	0.666	877.0	11133.0
62 GATGTTTTTGTCTGGTGTG	206	63.80	1.60	0.504	1.279	0.798	0.894	11503.0
63 ATGTTTTTTGTCTGGTGTGG	207	65.18			1.540	1.023	0.894	9492.8
64 TGTTTTTTGTCTGGTGTGGT	208	68.78	1.70	1.234	1.366	1.284	0.914	10704.0
65 стттттстстсствотод	209	68.28	1.70	1.161	1.366	1.239	0.933	10741.0
66 TTTTTGTCTGGTGTGAA	210	62.37	1.70	0.294	1.366	0.701	0.950	_
67 TTTTTGTCTGGTGTGGTAAG	211	62.23	1.70	0.273	1.366	0.689	0.941	7871.0
68 TTTTGTCTGGTGTGGTAAGT	212	65.28	1.20	0.721	0.931	0.801	0.921	7209.1
69 TTTGTCTGGTGTGGTAAGTC	213	99.99	1.20	0.908	0.931	0.917		
70 TTGTCTGGTGTGGTAAGTCC	214	70.25	0.30	1.449	0.149	0.955	1.022	7230.6
71 TGTCTGGTGTGGTAAGTCCC	215	73.77	-0.10	1.966	-0.199	1.143	866'0	6809.5
72 GTCTGGTGTGGTAAGTCCCC	216	77.74	-0.10	2.549	-0.199	1.504	0.913	7442.8
73 TCTGGTGTGGTAAGTCCCCA	217	75.28	-0.50	2.187	-0.547	1.148	0.824	2627.7
74 CTGGTGTGGTAAGTCCCCAC	218	74.18	-2.10	l	-1.938	0.519	182'0	
75 TGGTGTGGTAAGTCCCCACC	219	75.80	-3.50	2.263	-3.156	0.204	089'0	4182.3
76 GGTGTGGTAAGTCCCCACCT	220	77.89	-3.80	2.571	-3.417	0.296	0.518	474.7
77 GTGTGGTAAGTCCCCACCTC	221	77.05	-2.50	2.448	-2.286	0.649	67428	682.4
78 TGTGGTAAGTCCCCACCTCA	222	74.71	-2.50	2.105	-2.286	0.436	0.465	679.1
79 GTGGTAAGTCCCCACCTCAA	223	72.54	-2.10	1.785	-1.938	0.370	0.584	924.0
80 TGGTAAGTCCCCACCTCAAC	224	69.94	06.0-	1.404	-0.895	0.531	799'0	835.5



T. [1] (2) T. (2) T. (3) T. (5) T. (5) (1) T. (5) (1)

HIV PRT GeneChip™ Data		1213.6	1106.1	1009.0	1656.2	2178.3	2567.0	3000.5	2025.4	429.2	157.9	135.3	330.8	900.0	1177.0	795.1	889.2	1703.6	3115.2	4445.0	6762.8	8845.0	9010.6	19941.0	12577.0	7503.3	
		789.0	0.763	0.872	806.0	75	0.679	0.522	2	2	4	4	क्ट	97	3	77	14 24	78 7	0.598	0.847	1.070	1.227	1.253	1.260	1.257	1.149	
Window- Averaged Composite	Score	9	3	0.8	0.0	0.834	9-6	56	0.354	0.164	40.0-	445.0	-0.339	-0.370	008'0	-0.117	180.0	782.0	4	0.8	1.0	1.2	1.2	1.2	1.2	1.1	
Composite Score	1	0.772	1.037	0.874	0.788	0.970	1.132	0.779	0.240	-0.030	-0.223	-0.387	-0.363	-0.303	-0.639	-0.428	-0.249	0.272	0.889	1.023	1.141	1.539	1.314	1.314	1.368	1.071	
∆GMFOLD Score		-0.547	0.670	0.670	0.323	0.323	0.062	-0.199	-1.243	-1.851	-1.851	-1.851	-1.851	-1.851	-1.851	-1.851	-1.504	-0.199	-0.112	-0.112	0.062	0.670	0.583	0.583	0.583	0.583	
T _m Score		1.580	1.262	0.999	1.073	1.366	1.789	1.379	1.148	1.087	0.775	0.511	0.549	0.646	0.104	0.444	0.520	0.560	1	1.719	1.803	2.071	1.763	1.763	1.849	1.369	
∆G _{MFOLD} (kcal/mole @ 35 °C)	0	-0.50	06.0	06.0	0.50	0.50	0.20	-0.10	-1.30	-2.00	-2.00	-2.00	-2.00	-2.00	-2.00	-2.00	-1.60	-0.10	00.00		0.20	06.0	0.80	08.0	08.0	08.0	
RNA/DNA Tm (°C)	ì	/1.14	68.97	67.18	67.68	89.69	72.56	69.77	68.19	67.78	65.65	63.85	64.11	64.77	61.08	63.40	63.91	64.19	70.61	72.08	72.66	74.49	72.38	72.38	72.97	69.70	
SEQ ID NO:		225	226	227	228	229	230	231	232	233	234	235	236	237	238	239	240	241	242	243	244	245	246	247	248	249	-
DNA Probe Sequence	TO STREET STREET	81 GGTAAGTCCCCACCTCAACA	82 GTAAGTCCCCACCTCAACAG	83 TAAGTCCCCACCTCAACAGA	84 AAGTCCCCACCTCAACAGAT	85 AGTCCCCACCTCAACAGATG	86 GTCCCCACCTCAACAGATGT	87 TCCCCACCTCAACAGATGTT	88 CCCCACCTCAACAGATGTTG	89 CCCACCTCAACAGATGTTGT	90 CCACCTCAACAGATGTTGTC	91 CACCTCAACAGATGTTGTCT	92 ACCTCAACAGATGTTGTCTC	93 сстсаасадатетстса		95 TCAACAGATGTTGTCTCAGC	96 CAACAGATGTTGTCTCAGCT	97 AACAGATGTTGTCTCAGCTC	98 ACAGATGTTGTCTCAGCTCC	99 CAGATGTTGTCTCAGCTCCT	100 AGATGTTGTCTCAGCTCCTC	101 GATGTTGTCTCAGCTCCTCT	102 ATGTTGTCTCAGCTCCTCTA	103 TGTTGTCTCAGCTCCTCTAT	104 GTTGTCTCAGCTCCTCTATT	105 TIGICICAGCICCICIAITI	
p5 Probe Position	3	81	82	83	84	85	86	87	88	89	06	91	92	93	94	95	96	97	98	66	100	101	102	103	104	105	



T. [1] S. T. C. [1] " 11 2. [3] 12 [5] [1] -74-

		- (c)			∆GMFOLD	ပိ	Window-	HIV PRT
	Š	() E	(kcal/mole @ 35 °C)	Score	Score	Score	Averaged Composite Score	GeneChip™ Data
108 TCTCAGCTCCTCTATTTTG	252	66.57	0.80	0.910	0.583	0.786	0.942	7899 D
109 CTCAGCTCCTCTATTTTGT	253	68.39	08.0	1.177	0.583	0.952		
110 TCAGCTCCTCTATTTTGTT	254	69.99	08.0	0.927	0.583	0.796		
111 CAGCTCCTCTATTTTGTTC	255	69.99	08.0	0.927	0.583	0.796		
112 AGCTCCTCTATTTTTGTTCT	256	67.52	1.00	1.050	0.757	0.939		
113 GCTCCTCTATTTTGTTCTA	257	66.63	1.80	0.919	1.453	1.122		
114 CTCCTCTATTTTTGTTCTAT	258	62.13	1.80	0.259	1.453	0.713		
115 TCCTCTATTTTTGTTCTATG	259	59.97	1.80	-0.058	1.453	0.516		
116 CCTCTATTTTTGTTCTATGC	260	62.84	1.80	0.363	1.453	0.777	0.642	1824.5
117 CTCTATTTTTGTTCTATGCT	261	60.87	1.50	0.074	1.192	0.499		1169.2
118 TCTATTTTTGTTCTATGCTG	262	58.71	1.50	-0.244	1.192	0.302		683.7
119 CTATTTTTTTTTTTTGCTGC	263	61.60	1.50	0.181	1.192	0.565	0.765	1306.8
120 TATTTTTGTTCTATGCTGCC	264	63.53	1.50	0.464	1.192	0.741	0.834	2523.6
121 ATTTTGTTCTATGCTGCCC	265	96'.29	1.50	1.113	1.192	1.143		6682.0
122 TTTTTGTTCTATGCTGCCCT	266	96.69	1.50	1.407	1.192	1.325	1.060	9417.4
123 TTTTGTTCTATGCTGCCCTA	267	69.01	1.50	1.267	1.192	1.239	1.151	10339.0
124 TTTGTTCTATGCTGCCCTAT	268	68.62	1.50	1.210	1.192	1.203	1.254	10750.0
125 TTGTTCTATGCTGCCCTATT	269	68.62	1.50	1.210	1.192	1.203	1.282	11180.0
126 IGTTCTATGCTGCCCTATTT	270	68.62	1.50	1.210	1.192	1.203	1.271	11060.0
127 GTTCTATGCTGCCCTATTTC	271	70.37	1.80	1.468	1.453	1.462	1.221	16074.0
128 TTCTATGCTGCCCTATTTCT	272	00.69	1.80	1.266	1.453	1.337	1.144	9183.8
129 TCTATGCTGCCCTATTTCTA	273	68.05	1.80	1.127	1.453	1.251	1.082	8617.8
130 CTATGCTGCCCTATTTCTAA	274	64.38	1.70	0.589	1.366	0.884	1.040	7286.8
131 TATGCTGCCCTATTTCTAAG	275	62.71	1.50	0.344	1.192	0.666	0.978	3642.4
132 ATGCTGCCCTATTCTAAGT	276	66.39	08.0	0.883	0.583	0.769	0.883	3799.7
133 TGCTGCCCTATTTCTAAGTC	277	67.95	08.0	1.112	0.583	0.911	0.749	3408.3
134 GCTGCCCTATTTCTAAGTCA	278	69 25	Car	1 303	0.583	4 000	1100	1017



Table 4

p5 Probe Position	DNA Probe Sequence	SEQ ID NO:	RNA/DNA Tm (°C)	∆G _{MFOLD} (kcal/mole	T _m Score	∆GMFoLD Score	Composite Score	Window- Averaged	HIV PRT GeneChip™
				(© 32°C)				Composite Score	Data
135	135 стессстатттстаавтсав	279	65.26	08.0	0.718	0.583	0.667	0.536	2197.2
136	36 TGCCCTATTTCTAAGTCAGA	280	64.63	-0.10	0.626	-0.199	0.312	0.412	1125.0
137	137 GCCCTATTTCTAAGTCAGAT	281	64.73	-0.60	0.639	-0.634	0.156	0.244	1306.3
138	138 CCCTATTTCTAAGTCAGATC	282	61.98	09.0-	0.236	-0.634	-0.094	0.024	1019.5
39	139 CCTATTTCTAAGTCAGATCC	283	61.98	-0.60	0.236	-0.634	-0.094	0.120	1852.3
140	140 CTATTTCTAAGTCAGATCCT	284	60.05	09:0-	-0.046	-0.634	-0.270	-0.214	3159.3
141	141 TATTTCTAAGTCAGATCCTA	285	57.43	-0.60	-0.430	-0.634	-0.508	-0.284	2604.8
142	142 ATTTCTAAGTCAGATCCTAC	286	58.59	-0.60	-0.261	-0.634	-0.402	-0.315	3986.1
143	143 TTTCTAAGTCAGATCCTACA	287	59.91	-0.60	-0.068	-0.634	-0.283	-0.285	4500.7
144	144 TTCTAAGTCAGATCCTACAT	288	59.55	-0.60	-0.120	-0.634	-0.315	-0.233	4754.5
145	145 TCTAAGTCAGATCCTACATA	289	58.62	•	-0.257	-0.460	-0.334	-0.165	3802.1
146	146 CTAAGTCAGATCCTACATAC	290	57.80	1.20	-0.377	0.931	0.120	0.111	5069.4
147	147 TAAGTCAGATCCTACATACA	291	57.13	1.30	-0.476	1.018	0.092	0.059	3965.2
148	148 AAGTCAGATCCTACATACAA	292	55.78	,		1.018	-0.030	-0.031	3862.3
149	149 AGTCAGATCCTACATACAAA	293	55.78	1.30	-0.673	1.018	-0.030	0.020	2868.9
20	150 GTCAGATCCTACATACAAAT	294	55.62	1.70	-0.697	1.366	0.087	080-0	3542.9
151	151 TCAGATCCTACATACAAATC	295	54.02	1.50	-0.932	1.192	-0.125	0.122	2477.1
152	152 CAGATCCTACATACAAATCA	296	54.07	1.10	-0.924	0.844	-0.252	-0.081	2522.4
153	153 AGATCCTACATACAAATCAT	297	52.83	1.10	l	0.844	-0.365	-0.045	2554.6
154	154 GATCCTACATACAAATCATC	298	53.87	1.50	-0.953	1.192	-0.138	-0.034	3580.0
155	155 ATCCTACATACAAATCATCC	299	56.33	1.80	-0.591	1.453	0.185	-0.06	5937.7
156	156 TCCTACATACAATCATCCA	300	57.54	1.80	-0.415	1.453	0.295	-0.111	4606.7
157	157 CCTACATACAAATCATCCAT	301	56.32	1.80	-0.594	1.453	0.184	-0.159	4877.2
158	158 стасатасаватсятсятв	302	52.68	1.10	-1.128	0.844	-0.379	-0.278	2608.6
159	159 TACATACAAATCATCCATGT	303	53.56	0.30	-0.999	0.149	-0.563	-0.469	1491.7
160		304	53.56	-0.10	-0.999	-0.199	-0.695	-0.644	1364.3
161	161 CATACAAATCATCCATGTAT	305	53.07	-0.80	-1.071	-0.808	-0.971	-0.751	1089.8



Position	DNA Probe Sequence	NO:	RNA/DNA Tm (°C)	ΔG _{MFOLD} (kcal/mole @ 35 °C)	Score	∆GMFOLD Score	Composite Score	Window- Averaged Composite	HIV PRT GeneChip™ Data
								Score	
22	162 ATACAAATCATCCATGTATT	306	52.11	-1.10	-1.211	-1.069	-1.157	-0.818	1008.6
က္က		307	52.08	-0.40	-1.215	-0.460	-0.928		
7	164 ACAAATCATCCATGTATTGA	308	53.86	0.20	-0.955	0.062	-0.568	-0.921	535.8
35	165 CAAATCATCCATGTATTGAT	309	53.36	-0.50	-1.027	-0.547	-0.845		3
9	166 даатсатссатетаттвата	310	51.57	-0.70	-1.291	-0.721	-1.074		
<u></u>	167 AATCATCCATGTATTGATAG	311	53.47	-0.70	-1.012	-0.721	-0.901	0.685	
œ		312	56.66	-0.50	-0.543	-0.547	-0.545	0.700	165.2
6		313	56.66	-0.10	-0.543	-0.199	-0.412	-0.686	166.0
0	170 CATCCATGTATTGATAGATA	314	54.80	0.30	-0.817	0.149	-0.450		151.0
=	171 ATCCATGTATTGATAGATAA	315	51.69	0.30	-1.273	0.149	-0.733	0.621	101.8
7	172 TCCATGTATTGATAGATAAC	316	52.19	0.30	-1.199	0.149	-0.687	-0.724	84.0
က	173 CCATGIATTGATAGATAACT	317	52.89	0.30	-1.097	0.149	-0.623		
4	174 CATGTATTGATAGATAACTA	318	48.47	0.70	-1.746	0.496	-0.894	-0.937	67.8
S	175 ATGTATTGATAGATAACTAT	319	47.12	00.00	-1.944	-0.112	-1.248	-1.006	65.7
9	176 TGTATTGATAGATAACTATG	320	47.11	-0.20	-1.945	-0.286	-1.315	-1.048	0.06
~	177 GTATTGATAGATAACTATGT	321	49.90	-0.20	-1.536	-0.286	-1.061	-1.099	125.9
8	178 TATTGATAGATAACTATGTC	322	48.24	-0.20	-1.779	-0.286	-1.212	-1.083	132.6
စ	179 ATTGATAGATAACTATGTCT	323	50.78	-0.20	-1.407	-0.286	-0.981	-0.998	167.4
റ്റ		324	50.75	-0.20	-1.411	-0.286	-0.984	-0.916	219.0
Ξ	181 TGATAGATAACTATGTCTGG	325	53.01	-0.20	-1.080	-0.286	-0.778	-0.866	
2	182 gatagataactatgtctgga	326	54.36	-0.20	-0.881	-0.286	-0.655	-0.774	825.1
က္က	183 ATAGATAACTATGTCTGGAT	327	53.04	-0.10	-1.074	-0.199	-0.742	0.679	844.4
4	184 TAGATAACTATGTCTGGATT	328	53.37	-0.10	-1.027	-0.199	-0.712	0.569	912.6
Ŝ	185 AGATAACTATGTCTGGATTT	329	54.27	0.10	-0.895	-0.025	-0.565	0.448	1301.8
ø	186 GATAACTATGTCTGGATTTT	330	54.43	08.0	-0.870	0.583	-0.318	-0.335	1367.4
_	187 ATAACTATGTCTGGATTTTG	331	53.08	1.50	-1.070	1.192	-0.210	0.177	1284.2
<u></u>	188 TAACTATGTCTGGATTTTGT	332	56.05	1 50	-0 634	1 192	0.060	9000	1162 E

2002	DIAM Probe sequence		RNA/DNA	()		(
Position		 0 1	T _m (°C)	(kcal/mole	Score	∆GMFOLD Score	Score	Window- Averaged	HIV PRT GeneChip™
				(© 32 °C)				Composite Score	Data
189	189 AACTATGTCTGGATTTTGTT	333	56.97	1.50	-0 499	1 102	0 444	700	10001
190	190 ACTATGTCTGGATTTTGTTT	73.6	50 38		0.100	701.	0.144		1396.7
191	191 CTATGTCTGGATTTTGTTTT	205	09.00		-0.143	1.192	0.363		1348.3
192	100 TATGTCTGGATTTTTCTTTTTTTTTTTTTTTTTTTTTTT	222	01.RC		-0.177	1.192	0.343	0.264	1092.8
102	ATCHOOL TO THE TOTAL THE TENT OF THE TENT	336	57.45		-0.428	1.192	0.188	0.234	912.6
193		337	58.41	1.70	-0.287	1.366	0.341	0.123	994.3
194	194 IGICIGGAITITITA	338	57.81	2.00	-0.375	1.627	0.386	0.070	840 7
081		339	55.82	1.00	-0.667	0.757	-0.126	0.311	941 9
200		340	50.98	0.80	-1.377	0.583	-0.632	0.488	84 9
/AL		341	48.16	0.30	-1.790	0.149	-1.054	0.670	78.6
200	198 IGGATTTTTAAAAG	342	46.41	0.10	-2.048	-0.025	-1.279	-0.851	93.2
661	199 GGATTTTGTTTTAAAAGG	343	48.87	0.10	-1.686	-0.025	-1.055	-0.933	56.0
2007	200 GATTTTTTTAAAAGGC	344	50.22	0.10	-1.488	-0.025	-0.932	-0.912	49.9
107	201 ATTTGTTTTTAAAAGCT	345	50.84	0.10	-1.397	-0.025	-0.876	-0.843	55.0
707	ZUZ IIIIGIIIIIAAAAGGCTC	346	52.03	0.30	-1.223	0.149	-0.702	-0.768	646
203	ZU3 ITTGTTTTTAAAAGGCTCT	347	53.64	0.50	-0.987	0.323	-0.489	427.0	162.8
204	204 TTGTTTTTAAAAGGCTCTA	348	52.76	0.50	-1.115	0.323	-0.569	0.706	265.8
202	205 TGTTTTAAAAGGCTCTAA	349	50.71	0.50	-1.417	0.323	-0.756	0.677	288 5
206	206 GTTTTTAAAAGGCTCTAAG	350	50.86	0.50	-1.395	0.323	-0.742	0.672	548.4
707	20/ TTTTTAAAAGGCTCTAAGA	351	49.40	0.70	-1.609	0.496	-0.809	869.0	524 7
807	ZUB TTTTIAAAGGCTCTAAGAT	352	49.11	1.20	-1.651	0.931	-0.670	-0.746	937.9
507	ZUB ITTIAAAGGCTCTAAGATT	353	49.11	1.20	-1.651	0.931	-0.670	-0.790	1440 3
210	Z10 ITTAAAGGCTCTAAGATTT	354	49.11	1.20	-1.651	0.931	-0.670	-0.820	1633.3
117	Z11 I I AAAAGGCICIAAGATTTT	355	49.11	0.50	-1.651	0.323	-0.901	-0.735	1987 4
717	ZIZ IAAAGGCTCTAAGATTTTT	356	49.11	00.00	-1.651	-0.112	-1.067	-0.627	1792.3
4137	213 AMANGGCICIAAGATTTTG	357	49.63	0.20	-1.575	0.062	-0.953	-0.495	2218.9
214,		358	54.13		-0.914	0.931	-0.213	-0.365	2371.4
7017	AGGCICI HAGAIIIIIGIC	329	57.38	1.20	-0.439	0.931	0.082	0.028	0 0000



DNA Probe Sequence SE	ω Σ	SEQ ID NO:	RNA/DNA Tm (°C)	∆G _{MFOLD} (kcal/mole @ 35 °C)	Score	∆G _{MFOLD} Score	Composite Score	Window- Averaged Composite Score	HIV PRT GeneChip™ Data
216 AGGCTCTAAGATTTTTGTCA 360	360	4	60.78	08.0	0.061	0.583	0.260	280'0	4070.5
217 GGCTCTAAGATTTTTGTCAT 361	361		60.56	08.0	0.028	0.583	0.239	0.048	5394.5
218 GCTCTAAGATTTTTGTCATG 362	362		57.81	08.0	-0.376	0.583	-0.011	150'0	2025.5
219 CTCTAAGATTTTTGTCATGC 363	363		57.81	08.0	-0.376	0.583	-0.011	900'0	1741.9
220 TCTAAGATTTTTGTCATGCT 364	364	L	57.81	08.0	-0.376	0.583	-0.011		1707.6
221 CTAAGATTTTTGTCATGCTA 365	365		55.87	08.0	-0.660	0.583	-0.187		
222 TAAGATTTTTGTCATGCTAC 366	366		54.43	08.0	-0.872	0.583	-0.319	920'0	3131.4
223 AAGATTTTGTCATGCTACT 367	367	<u> </u>	56.99	09'0	-0.495	0.410	-0.151		4892.5
224 AGATTTTTGTCATGCTACTT 368	368		59.39	09'0	-0.144	0.410	0.067	-	5856.4
225 GATTTTGTCATGCTACTTT 369	369		59.54	09.0	-0.122	0.410	080'0		6439.0
226 ATTTTGTCATGCTACTTTG 370	370		58.09	09.0	-0.334	0.410	-0.051		5820.3
227 TTTTTGTCATGCTACTTTGG 371	371		60.78	09.0	0.060	0.410	0.193		5189.6
228 TTTTGTCATGCTACTTTGGA 372	372		61.79	09.0	0.209	0.410	0.285		4721.7
229 TTTGTCATGCTACTTTGGAA 373	373	<u>. </u>	59.35		1				
230 TIGICAIGCTACTIIGGAAI 374	374		59.00	09'0	-0.200	0.410	0.032		
231 TGTCATGCTACTTTGGAATA 375	375		58.10	09'0	-0.333	0.410	-0.051	0.00.0	4102.0
232 GTCATGCTACTTTGGAATAT 376	376		58.16	06'0	-0.324	0.670	0.054		
233 TCATGCTACTTTGGAATATT 377	377		55.52	06'0	-0.711	0.670	-0.186	·	2407.9
_	378.	<u> </u>	54.23	1.30			•		
235 ATGCTACTTTGGAATATTGC 379	379		56.90	1.40			0.105	-	
236 TGCTACTTTGGAATATTGCT 380	380		58.82	06.0	-0.227	0.670	0.114		1894.1
237 GCTACTTTGGAATATTGCTG 381	381		58.82	1.30			0.246		
238 CTACTTTGGAATATTGCTGG 382	382		57.35	1.70	-0.443	1.366	0.244	0.270	1363.0
239 TACTTTGGAATATTGCTGGT 383	383		58.39	1.70	-0.290	1.366	0.339	0.299	1217.5
240 ACTTTGGAATATTGCTGGTG 384	384		58.88	1.70	-0.217	1.366	0.384		1621.8
241 CTTTGGAATATTGCTGGTGA 385	385		59.64	1.70	-0.106	1.366	0.453	0.346	1438.2
242 TTTGGAATATTGCTGGTGAT 386	386	<u>. </u>	57.72	1.80	-0.388	1.453	0.311	0.345	1608.0

HIV PRT	Genechip ''' Data	2334.6	3776.7	5648.7	5358.8	5517.2	6246.4	9975.1	11990.0	11543.0	14125.0	23489.0	15975.0	16053.0	19205.0	17872.0	12871.0	8792.7	9.6099	3018.0	1802.6	1074.0	1132.5	1454.5	1676.8	2268.9	1682.6	
	Averaged Gene Composite D Score	0.302	0.244	0.216	0.261	0.316	0.368	0.444	0.599	0.756	0.862	0.936	1.022	1.082	1.136	1.080	0.955	0.800	0.653	0.454	908-0	0.205	0.120	0.074	0.032	97070	4000	
ite	Score Proposition Av	0.403	0.288	0.239	0.039	-0.049	0.279	0.629	0.788	0.655	0.770	1.119	1.052	1.019	1.151	1.391	1.072	1.151	0.721	0.181	-0.001	0.054	-0.018	0.067	0.430	0.125	-0.160	
_	Score	1.453	0.323	-0.286	-0.286	-0.286	-0.286	-0.286	-0.286	-0.634	-0.634	-0.634	-0.634	-0.721	-0.373	0.062	0.062	-0.373	-1.504	-2.373	-2.547	-2.547	-2.547	-2.373	-1.591	-2.025	-2.025	
	Score	-0.241	0.266	0.561	0.238	0.097	0.625	1.190	1.446	1.446	1.631	2.193	2.085	2.085	2.085	2.206		2.085	2.085		1.559	1.648		1.562	1.669	1.443	0.983	
∆GMFOLD	(Kcal/mole @ 35 °C)	1.80	0.50	-0.20	-0.20	-0.20	-0.20	-0.20	-0.20	-0.60	-0.60	-0.60	-0.60	-0.70	-0.30	0.20	0.20	-0.30	-1.60	-2.60	-2.80	-2.80	-2.80	-2.60	-1.70	-2.20	-2.20	
RNA/DNA	() E	58.73	62.18	64.19	61.99	61.03	64.63	68.48	70.22	70.22	71.48	75.32	74.58	74.58	74.58	75.40	71.89	74.58	74.58	72.27	71.00	71.60	70.81	71.02	71.74	70.20	67.07	
SEQ ID	5	387	388	389	390	391	392	393	394	395	396	397	398	399	400	401	402	403	404	405	406	407	408	409	410	411	412	
DNA Probe Sequence		243 TTGGAATATTGCTGGTGATC	244 TGGAATATTGCTGGTGATCC	245 GGAATATTGCTGGTGATCCT	246 GAATATTGCTGGTGATCCTT		248 ATATTGCTGGTGATCCTTTC	249 ratrecreerearcerree	250 ATTGCTGGTGATCCTTTCCA			253 gcrggrgarccrtrccarcc	254 cregrearccrrrccarcc	255 regrearcerrrecareeer	256 gergarccrirccarccrg	257 grearccrrrccarccrgr	258 rgarcctrrccarccrgrg	259 garcerrecarecerge	260 ATCCTTTCCATCCCTGTGGA	261 rccttrccarccrgrggaa		263 CTTTCCATCCCTGTGGAAGC	264 TTTCCATCCTGTGGAAGCA	265 TTCCATCCTGTGGAAGCAC	266 rccarccrgrggaagcaca	267 CCATCCCTGTGGAAGCACAT	268 CATCCCTGTGGAAGCACATT	CHE LO LOS LA LOS COMPANIONS CONTRACTOR CONT
p5 Probe		243	244	245	246	247	248	249	250	251	252	253	254	255	256	257	258	259	260	261	292	263	264	265	266	267	268	000

	DNA Probe Sequence	SEQ ID	RNA/DNA	AGMFOLD		∆GMFOLD	Composite	Window-	HIV PRT
rosition		ö	ري ۳ (در)	(kcal/mole @ 35 °C)	Score	Score	Score	Averaged Composite Score	GeneChip™ Data
270 TCC	270 recerenceaageacatrer	414	68.98	-2.20	1.263	-2.025	0.014	0220	12818
271 ccc	271 CCCTGTGGAAGCACATTGTA	415	66.92		0.962	-2.025	-0.173		
272 ccr	272 CCTGTGGAAGCACATTGTAC	416	63.84		0.509	-2.025	-0.454		7003
273 CTG	273 CTGTGGAAGCACATTGTACT	417	62.01	-2.20	0.241	-2.025	-0.620		618.7
274 TGT	274 TGTGGAAGCACATTGTACTG	418	59.99	-2.00	-0.056	-1.851	-0.738		
275 GTG	275 GTGGAAGCACATTGTACTGA	419	61.39	•	0.149	-0.547	-0.115	-0.347	
276 TGG	276 TGGAAGCACATTGTACTGAT	420	58.35	0.50	-0.296	0.323	-0.061	-0.331	
2// GGA	2// GGAAGCACATTGTACTGATA	421	57.86	09'0	-0.368	0.323	-0.106	0.230	1314.7
278 GAA	278 GAAGCACATTGTACTGATAT	422	55.32		-0.740	0.323	-0.336	0.141	1102.5
279 AAG	2/9 AAGCACATTGTACTGATATC	423	55.30	0.50	-0.744	0.323	-0.339	0.200	1222.1
280 AGC	280 AGCACATTGTACTGATATCT	424	59.26	0.50	-0.162	0.323	0.022		1893.2
281 GCA	281 GCACATTGTACTGATATCTA	425	58.48	0.50	-0.277	0.323	-0.049		2097.7
282 CAC	282 CACATTGTACTGATATCTAA	426	52.51	0.50	-1.152	0.323	-0.592	0.446	1237.8
283 ACA	283 ACATTGTACTGATATCTAAT	427	51.20	0.50	-1.345	0.323	-0.711	0.443	959.5
284 CAT	284 CATTGTACTGATATCTAATC	428	51.89	0.10	-1.244	-0.025	-0.781	0.472	
285 ATT	285 ATTGTACTGATATCTAATCC	429	54.53	-0.30	-0.856	-0.373	-0.672	0.490	
286 TTG	286 TTGTACTGATATCTAATCCC	430	58.41	-0.30	-0.287	-0.373	-0.320	0.436	4191.6
287 TGT	287 IGTACTGATATCTAATCCCT	431	66'69	-0.30	-0.055	-0.373	-0.176	0.320	5565.8
288 GTA	288 GTACTGATATCTAATCCCTG	432	66.65	-0.30	-0.055	-0.373	-0.176	0.202	9980.2
289 TAC	289 TACTGATATCTAATCCCTGG	433	59.52	-0.30	-0.124	-0.373	-0.218	0.084	6318.9
290 ACT	290 ACTGATATCTAATCCCTGGT	434	63.07	-0.30	0.397	-0.373	0.104	0.023	7749.5
291 CTG	291 CTGATATCTAATCCCTGGTG	435	62.43	-0.30	0.303	-0.373	0.046	0.184	8165.3
292 TGA	292 TGATATCTAATCCCTGGTGT	436	09.60	-0.30	0.474	-0.373	0.152	0.365	9107.6
293 GAT	293 GATATCTAATCCCTGGTGTC	437	62.19	0.10	0.707	-0.025	0.429	0.566	13914.0
294 ATA	294 ATATCTAATCCTGGTGTCT	438	65.82	1.50	0.800	1.192	0.949	869'0	15093.0
295 TAT		439	67.41	1.50	1.033	1.192	1.093	0.822	18647.0
296 ATC	296 ATCTAATCCCTGGTGTCTCA	440	69.20	1.30	1.296	1.018	1.190	0.904	21810.0

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HIV PRT GeneChip™ Data	20102.0	20967.0	18200.0	19845.0	19231.0	17629.0	17009.0	11580.0	8374.6	6153.3	7134.0	4435.2	2035.5	2466.6	1080.9	956.0	529.4	471.4	510.4	531.0	613.3	685.1	300.0	316.1	387.5	685.7	759.6
Window- Averaged Composite Score	966.0	1.052	1.092	1.088	1.057	0.981	0.918	0.800	009.0	0.355	0.177	0.050	0.043	0.148	-0.268	0.325	-0.302	0.328	0.389	-0.486	099'0	0.620	0.630	-0.672	9990	7990	-0.430
Composite Score	1.025	0.914	0.725	1.077	1.336	1.375	1.164	0.808	0.386	0.281	0.252	-0.062	-0.342	-0.082	-0.082	-0.269	-0.458	-0.583	-0.463	-0.176	-0.264	-0.510	-0.947	-0.975	-1.004	-0.594	-0.411
∆GMFOLD Score	0.583	0.583	0.583	0.583	0.583	0.583	0.583	0.583	0.583	0.670	0.062	0.062	0.062	0.062	0.062	0.062	0.062	0.062	0.062	0.062	0.062	-0.112	-0.112	-0.112	-0.112	-0.112	0.062
Score	1.296	1.117	0.811	1.380	1.797	1.860	1.519	0.946	0.264	0.042	0.369	-0.138	-0.589	-0.170	-0.170	-0.472	-0.776	-0.978	-0.785	-0.322	-0.463	-0.753	-1.459	-1.504	-1.551	-0.889	-0.700
∆G _{MFOLD} (kcal/mole @ 35 °C)	08.0	08.0	0.80	08.0	08.0	08.0	08.0	08.0	08.0	06.0	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	00.00	00.00	00.00	00.00	00.00	0.20
RNA/DNA Tm (°C)	69.20	67.98	65.90	69.78	72.61	73.04	70.72	66.82	62.17	60.65	62.88	59.43	56.35	59.21	59.21	57.15	55.08	53.70	55.01	58.17	57.21	55.23	50.45	50.12	49.79	54.30	55.59
SEQ ID NO:	441	442	443	444	445	446	447	448	449	450	451	452	453	454	455	456	457	458	459	460	461	462	463	464	465	466	467
DNA Probe Se			299 TAATCCCTGGTGTCTCATTG	300 AATCCCTGGTGTCTCATTGT	301 ATCCCTGGTGTCTCATTGTT				305 CTGGTGTCTCATTGTTTATA		307 GGTGTCTCATTGTTTATACT		309 TGTCTCATTGTTTATACTAG	310 GTCTCATTGTTTATACTAGG	311 TCTCATTGTTTATACTAGGT	312 CTCATTGTTTATACTAGGTA	313 TCATTGTTTATACTAGGTAT	314 CATTGTTTATACTAGGTATG	315 ATTGTTTATACTAGGTATGG	316 TTGTTTATACTAGGTATGGT	317 TGTTTATACTAGGTATGGTA				321 TATACTAGGTATGGTAAATG	322 ATACTAGGTATGGTAAATGC	323 TACTAGGTATGGTAAATGCA
p5 Probe Position	29	29	29	င္က	8	င္တ	30	S	8	8	30	30	30	31	31	31	31	31	31	31	31	31	31	32	32	32	32



F.C		1050.2	1020.4	742.6	889.6	858.8	379.0	166.7	215.3	103.2	246.3	163.4	294.1	531.4	1995.5	510.1	555.4	214.0	825.7	1582.6	2391.8	2276.3	2702.8	31.7	5343.4	6309.0	72.4	3835.3
HIV PRT GeneChip™ Data		105	102	74	88	85	37	16	21	10	24	16	29	53	199	51	55	121	82	158	236	227	270	3781.7	534	930	6372.	
Window- Averaged Composite	Score	0.294	-0.157	-0.109	0.132	-0.182	-0.262	-0.257	0.249	-0.303	-0.326	-0.303	-0.264	0.220	0.233	0.279	-0.264	-0.238	282.0-	-0.261	_	-0.273	-0.252	-0.274	-0.314		-0.332	-0.369
Composite Score		-0.146	0.177	-0.054	-0.002	-0.070	-0.256	-0.575	-0.495	-0.380	-0.021	0.055	-0.448	-0.415	-0.415	-0.221	-0.134		-0.263	-0.348	-0.232	-0.409	-0.389	-0.254	-0.014		-0.500	-0.513
∆GMFOLD Score		0.583	0.844	0.844	0.844	0.844	0.844	0.236	0.236	0.236	0.236	0.583	-0.025	-0.373	-0.373	-0.373	-0.895	l	-1.069	-1.069	-1.069	-1.069	-1.156	-1.156	-1.156	-1.156	-1.156	-1.156
Score		-0.593	-0.232	-0.605	-0.521	-0.631	-0.931	-1.071	-0.943	-0.757	-0.178	-0.269	-0.708	-0.441	-0.441	-0.128	0.332		0.230	0.094	0.281	-0.004	0.080	0.299	0.686	0.512	-0.098	0.120
∆G _{MFOLD} (kcal/mole @ 35 °C)		08.0	1.10	1.10	1.10	1.10	1.10	0.40	0.40	0.40	0.40	08.0	0.10	-0.30	-0.30	-0.30	06:0-	-1.10	-1.10	-1.10	-1.10	-1.10	-1.20	-1.20	-1.20	-1.20	-1.20	-1.20
RNA/DNA Tm (°C)		56.32	58.78	56.24	56.81	56.07	54.02	53.06	53.94	55.21	59.15	58.53	55.54	57.36	57.36	59.50	62.63	64.24	61.94	61.00	62.28	60.34	60.91	62.40	65.05	63.86	59.70	59.55
SEQ ID NO:		468	469	470	471	472	473	474	475	476	477	478	479	480	481	482	483	484	485	486	487	488	489	490	491	492	493	494
DNA Probe Sequence		324 ACTAGGTATGGTAAATGCAG	325 CTAGGTATGGTAAATGCAGT	326 TAGGTATGGTAAATGCAGTA	327 AGGTATGGTAAATGCAGTAT	328 GGTATGGTAAATGCAGTATA	329 GTATGGTAAATGCAGTATAC	330 TATGGTAAATGCAGTATACT	331 ATGGTAAATGCAGTATACTT	332 TGGTAAATGCAGTATACTTC	333 GGTAAATGCAGTATACTTCC	334 GTAAATGCAGTATACTTCCT	335 TAAATGCAGTATACTTCCTG	336 AAATGCAGTATACTTCCTGA	337 AATGCAGTATACTTCCTGAA	338 ATGCAGTATACTTCCTGAAG	339 TGCAGTATACTTCCTGAAGT	340 GCAGTATACTTCCTGAAGTC	341 CAGTATACTTCCTGAAGTCT	342 AGTATACTTCCTGAAGTCTT	343 GTATACTTCCTGAAGTCTTC	344 TATACTTCCTGAAGTCTTCA	345 ATACTTCCTGAAGTCTTCAT	346 TACTTCCTGAAGTCTTCATC	347 ACTTCCTGAAGTCTTCATCT	348 CTTCCTGAAGTCTTCATCTA	349 TTCCTGAAGTCTTCATCTAA	350 TCCTGAAGTCTTCATCTAAG
p5 Probe Position		324	325	326	327	328	329	330	331	332	333	334	335	336	337	338	339	340	341	342	343	344	345	346	347	348	349	350

HIV PRT	GeneChip [™] Data	8925.5	1211.8	609.4	629.1	749.3	805.6	817.0	327.1	320.0	84.1	67.7	62.2	78.9	43.2	50.4	43.7	45.6	49.8	53.2	56.6	45.3	56.3	61.7	224.5	413.0	15010
Window-	Averaged Composite Score	-0.423	-0.472	4440	-0.358	0.344	0.374	0.449	983.0	-0.645	-0.822	-1.002	-1.171	-1.298	-1.328	-1.322	-1.242	-1.163	-1.119	-1.082	-0.958	-0.844	-0.773	-0.702	-0.616	-0.476	0000
ပိ	Score	-0.404	-0.520	-0.512	-0.390	-0.461	-0.098	-0.124	-0.281	-0.733	-1.057	-0.998	-1.227	-1.336	-1.382		-1.616	-1.267	-0.958	-0.670	-0.783	-1.076	-1.206	-0.746	-0.471	-0.455	0.170
∆G мго∟р	Score	-1.156	-1.156	-0.982	-0.199	-0.199		-0.199	-0.199	-0.634	-0.634	-0.025	-0.025	-0.025	-0.025		-0.025	-0.025	0.149	0.931	0.931	0.410	0.236	0.236	0.236	0.410	1 018
۳		0.057	-0.130	-0.224	-0.507	-0.622	-0.036	-0.077	-0.331	-0.794	-1.316	-1.595	-1.963	-2.140	-2.214	-2.349	-2.591	-2.028		-1.651	-1.834	-1.987	-2.090	-1.347	-0.905	-0.985	-0 913
∆GMFOLD	(kcal/mole @ 35 °C)	-1.20	-1.20	-1.00	-0.10	-0.10	-0.10	-0.10	-0.10	-0.60	-0.60	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.30	1.20	1.20	09.0	0.40	0.40	0.40	09.0	1.30
RNA/DNA	T _m (°C)	92.09	59.48	58.84	56.91	56.13	60.12	59.84	58.11	54.95	51.39	49.50	46.98	45.78	45.27	44.36	42.71	46.54	49.21	49.11	47.87	46.82	46.12	51.18	54.20	53.65	54 14
SEQ ID	Ö	495	496	497	498	499	200	501	502	503	504	505	909	507	508	509	510	511	512	513	514	515	516	212	518	619	065
DNA Probe Sequence		351 CCTGAAGTCTTCATCTAAGG	352 CTGAAGTCTTCATCTAAGGG	353 TGAAGTCTTCATCTAAGGGA	354 GAAGTCTTCATCTAAGGGAA	355 AAGTCTTCATCTAAGGGAAC	356 AGTCTTCATCTAAGGGAACT	357 GTCTTCATCTAAGGGAACTG	358 TCTTCATCTAAGGGAACTGA	359 CTTCATCTAAGGGAACTGAA	360 TTCATCTAAGGGAACTGAAA	361 TCATCTAAGGGAACTGAAAA	362 CATCTAAGGGAACTGAAAA	363 ATCTAAGGGAACTGAAAAT	364 TCTAAGGGAACTGAAAATA	365 CTAAGGGAACTGAAAATAT	366 TAAGGGAACTGAAAATATG	367 AAGGGAACTGAAAAATATGC		369 сесаастеаааалатесат	370 GGAACTGAAAAATATGCATC	371 GAACTGAAAAATATGCATCA	372 AACTGAAAATATGCATCAC		374 CTGAAAATATGCATCACCC	375 TGAAAATATGCATCACCCA	376 GAAAATATGCATCACCCAC
p5 Probe	Position	351	352	353	354	355	356	357	358	329	360	361	362	363	364	365	366	367	368	369	370	371	372	373	374	375	376

2	22	
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55.78		522. 55
58.72	2	523 5 8
64.13	9	524 6
67.27	9	525 6
67.53		526
71.21		527
70.68		528
71.39		529
69.16		530
67.91		531
71.15		532
69.94	U	533 6
68.25	9	
68.25	19	535 6
99.50	9	536 6
62.61	9	537 6
62.71	9	538 (
62.12	9	539 6
61.21	9	540 6
61.58	9	541 6
60.48	9	542 6
56.84	5	543 5
55.82	5	544 5
57.04	9	545 5
55.95	S.	546 5
56.89	2	547 5
26.67	2	548 5



	SEQ ID NO:	RNA/DNA Tm (°C)	ΔG _{MFOLD} (kcal/mole @ 35 °C)	Score	∆GMFoLD Score	Composite Score	Window- Averaged Composite	HIV PRT GeneChip™ Data
405 TGTTACTGATTTTTCTTTT	549	54.96	-0.10	-0.793	-0.199	-0.567	Score <u>0.575</u>	3923.2
406 GTTACTGATTTTTTTTTTT	550	55.36		-0.734	-0.199	-0.531	-0.646	4093.5
407 TTACTGATTTTTTTTTT	551	52.62	-0.10	-1.136	-0.199	-0.780	0.730	1381.5
408 TACTGATTTTTTTTTA	552	51.70	-0.10	-1.272	-0.199	-0.864	-0.784	1194.3
409 ACTGATTTTTTTTTAA	553	50.45	-0.10	-1.454	-0.199	-0.977	-0.746	2371.3
410 CTGATTTTTTTTTAAC	554	50.45	-0.10	-1.454	-0.199	-0.977	0.682	395.9
411 TGATTTTTTTTTAACC	555	52.50	-0.10	-1.155	-0.199	-0.792	-0.583	230.7
	556	56.43	0.30	-0.578	0.149	-0.302	-0.423	314.9
413 ATTTTTTTTTTAACCCT	557	57.05	08.0	-0.487	0.583	-0.080	-0.246	276.
414 TTTTTTTTTTAACCCTG	558	56.99	08.0	-0.495	0.583	-0.085	-0.046	273.3
415 TTTTTTTTTAACCCTGC	559	89.09	08.0	0.045	0.583	0.250	200.0	628.4
416 TTTTTTTTAACCCTGCG	260	60.85	08.0	0.071	0.583	0.265	0.155	4661.4
417 TTTCTTTTTAACCCTGCGG	561	62.93	0.70	0.377	0.496		0.167	411.2
418 TTCTTTTTAACCCTGCGGG	562	65.01	09'0-	0.681	-0.634	0.181	0.156	289.5
419 TCTTTTTAACCCTGCGGA	563	65.91	-1.00	0.813	-0.982	0.131	0.130	244.8
420 CTTTTTAACCCTGCGGGAT	564	64.52	-1.00	0.610	-0.982	0.005	980'0	250.7
421 TTTTTAACCCTGCGGGATG	565	62.66	-1.00	0.337	-0.982	-0.164	290'0	207.8
422 TTTTTAACCCTGCGGGATGT	999	65.23	-1.00	0.713	-0.982	0.069	0.106	255.8
423 TTTTAACCCTGCGGGATGTG	567	64.80	-1.00	0.651	-0.982	0.030	0.142	356.8
424 TTTAACCCTGCGGGATGTGG	568	66.83	-1.00	0.949	-0.982	0.215	0.201	497.8
425 TTAACCCTGCGGGATGTGGT	569	69.50	-1.00	1.339	-0.982	0.457	0.318	754.3
426 TAACCCTGCGGGATGTGGTA	570	68.63	-1.00	1.212	-0.982	0.378	0.434	905.
427 AACCCTGCGGGATGTGGTAT	571	69.14	-1.00	1.286	-0.982	0.424	0.555	1186.6
428 ACCCTGCGGGATGTGGTATT	572	71.66	-1.00	1.657	-0.982	0.654	905'0	1514.9
	573	72.66	-0.60	1.804	-0.634	0.878	0.569	2407.6
430 ccrecegeareregrarrec	574	72.66	09.0-	1.804	-0.634	0.878	0.526	3019.4
431 CTGCGGGATGTGGTATTCCT	575	71.02	-1.30	1.563	-1.243	0.497	0.426	3275.3



ns Prohe	DNA Probe Sequence	SEQ ID	RNA/DNA	∆GMFOLD	, E	∆GMFOLD	Composite	Window-	ואין אווו
Position		Ö	T _m (°C)	(kcal/mole @ 35 °C)	a.	Score	Score	Averaged Composite Score	GeneChip™ Data
432	432 TGCGGGATGTGGTATTCCTA	576	68.54	-1.30	1.199	-1.243	0.271	0.294	2830.8
433	433 GCGGGATGTGGTATTCCTAA	577	66.48	-1.30	0.896	-1.243	0.083	0.108	2620.5
434	434 CGGGATGTGGTATTCCTAAT	578	62.46	-1.30	0.307	-1.243	-0.282	0.058	1827.8
435	435 GGGATGTGGTATTCCTAATT	579	62.37	-1.30	0.294	-1.243	-0.290		1957.4
436	436 GGATGTGGTATTCCTAATTG	580	59.71	06.0-	-0.097	-0.895	-0.400		,
437	437 GATGTGGTATTCCTAATTGA	581	58.45	-0.20	-0.281	-0.286	-0.283		
438	438 ATGTGGTATTCCTAATTGAA	582	55.24	-0.20	-0.752	-0.286	-0.575		1245.7
439	439 TGTGGTATTCCTAATTGAAC	583	55.76	-0.30	-0.675	-0.373	-0.561	-0.473	
440	440 GTGGTATTCCTAATTGAACT	584	57.73	-0.30	-0.387	-0.373			
441	441 TGGTATTCCTAATTGAACTT	585	55.15	-0.30	-0.765	-0.373	-0.616		
447	442 GGTATTCCTAATTGAACTTC	586	56.47	-0.30	-0.572	-0.373	-0.496		
44	443 GTATTCCTAATTGAACTTCC	587	57.76	-0.30	-0.383	-0.373			
447	444 TATTCCTAATTGAACTTCCC	588	58.57	-0.30	-0.264	-0.373	-0.306		
446	445 ATTCCTAATTGAACTTCCCA	589	60.26	-0.30	١.	-0.373			
446	446 TTCCTAATTGAACTTCCCAG	590	60.45	-0.10	0.013	-0.199			
44	447 TCCTAATTGAACTTCCCAGA	591	61.36	0.70	0.146	0.496	0.279		
448	448 CCTAATTGAACTTCCCAGAA	592	58.27	0.70	-0.308	0.496			_
446	449 CTAATTGAACTTCCCAGAAG	593	54.92	-0.70	-0.800	-0.721	-0.770		
45(450 TAATTGAACTTCCCAGAAGT	594	55.84	-1.90	-0.664	-1.764	-1.082	,	
45	451 AATTGAACTTCCCAGAAGTC	595	57.61	-2.10	L				
45	452 ATTGAACTTCCCAGAAGTCT	596	61.42	-2.10	0.154	-1.938	-0.641		
45	453 TTGAACTTCCCAGAAGTCTT	597	61.76	5 -2.10	0.205	-1.938			
45,	454 TGAACTTCCCAGAAGTCTTG	598	61.34	1 -2.10	0.143	-1.938			
45	455 GAACTTCCCAGAAGTCTTGA	599	62.71	1 -2.10	0.344	-1.938	-0.523		
45	456 AACTTCCCAGAAGTCTTGAG	009	61.63	3 -2.10	0.186	-1.938			
45	457 ACTTCCCAGAAGTCTTGAGT	601	66.97	1.90	0.969	-1.764	690'0-		
45	458 CTTCCCAGAAGTCTTGAGTT	602	66.75	5 -1.00	0.937	-0.982	0.208	3 0.019	736.1



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HIV PRT GeneChip™ Data	815.	888.8	2021.6	1988.5	2008.8	2631.8	3052.8	3509.3	3221.6	3677.1	1176.6	1168.	666.3	674.0	1471.4	1493.5	2191.5	1410.3		-			0.607				2511.5
Window- Averaged Composite Score	0.058	0.101	0.049	210.0-	0.082	-0.105	-0.151	0.212				-0.255	-0.292			0.240	7770-	0.317			0.629	989'0'	-0.768	-0.775	-0.732		-0.646
Composite Score	0.432	0.253	0.187	0.017	-0.319	-0.434	-0.223	-0.057	0.091	-0.132	-0.409	-0.312	-0.471		-0.173	-0.169	0.011	-0.301	-0.204		-0.866	-0.841	-0.912	-0.762	-0.762	-0.716	-0.567
∆GMFOLD Score	-0.286	-1.156	-1.330	-1.330	-1.330	-1.156	-0.547	0.149	0.149	0.149	0.149	-0.025	-0.025	-0.025	-0.025	-0.025	-0.025	-0.025	-0.025	-0.025	-0.025	0.323	0.323	0.323	0.323	0.323	0.323
Score	0.872	1.116	1.116	0.842	0.300	0.009	-0.025	-0.183	0.056	-0.305	-0.751	-0.488	-0.745	-0.589	-0.263	-0.257	0.032	-0.471	-0.314	-0.817	1.382	1.554	-1.668	1.427	1.427	1.352	1.112
∆G _{MFOLD} (kcal/mole @ 35 °C)	-0.20	-1.20	-1.40	-1.40	-1.40	-1.20	-0.50	0.30	0.30	0.30	0.30	0.10	0.10	0.10	0.10	0.10	0.10		0.10	0.10	0.10	0.50	0.50		0.50	0.50	0.50
RNA/DNA Tm (°C)	66.31	67.98	67.98	66.10	62.41	60.43	60.20	59.12	60.75	58.29	55.25	57.04	55.29	56.35	58.57	58.61	60.59	57.16	58.23	54.79	50.95	49.77	48.99	50.64	50.64	51.15	52.79
SEQ ID NO:	603	604	605	909	607	608	609	610	611	612	613	614	615	616	617	618	619	620	621	622	623	624	625	626	627	628	629
DNA Probe Sequence	459 TTCCCAGAAGTCTTGAGTTC	460 TCCCAGAAGTCTTGAGTTCT	461 CCCAGAAGTCTTGAGTTCTC	462 CCAGAAGTCTTGAGTTCTCT	463 CAGAAGTCTTGAGTTCTCTT	464 AGAAGTCTTGAGTTCTCTTA	465 GAAGTCTTGAGTTCTCTTAT	466 AAGTCTTGAGTTCTCTTATT	467 AGTCTTGAGTTCTCTTATTA	468 GTCTTGAGTTCTCTTATTAA	469 TCTTGAGTTCTCTTATTAAG	470 CTTGAGTTCTCTTATTAAGT	471 TTGAGTTCTCTTATTAAGTT	472 TGAGTTCTCTTATTAAGTTC	473 GAGTTCTCTTATTAAGTTCT	474 AGTTCTCTTATTAAGTTCTC	475 GTTCTCTTATTAAGTTCTCT	476 ITCTCTTATTAAGTTCTCTG	477 TCTCTTATTAAGTTCTCTGA	478 CTCTTATTAAGTTCTCTGAA	479 TCTTATTAAGTTCTCTGAAA	480 CTTATTAAGTTCTCTGAAAT	481 TTATTAAGTTCTCTGAAATC	482 TATTAAGTTCTCTGAAATCT	483 ATTAAGTTCTCTGAAATCTA	484 TTAAGTTCTCTGAAATCTAC	485 TAAGTTCTCTGAAATCTACT
p5 Probe Position	459	460	461	462	463	464	465	466	467	468	469	470	471	472	473	474	475	476	477	478	475	480	481	48%	48	484	484

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po Probe Position	DNA Probe Sequence	SEQ ID NO:	RNA/DNA Tm (°C)	∆G _{MFOLD} (kcal/mole @ 35 °C)	Score	∆GMFOLD Score	Composite Score	Window- Averaged Composite	HIV PRI GeneChip™ Data
540	540 GGCAAATACTGGAGTATTGT	684	58.33	-1.20	-0.298	-1.156	-0.624		435.3
541	541 GCAAATACTGGAGTATTGTA	685	55.24	-1.20	-0.753	-1.156		-0.974	
542	542 CAAATACTGGAGTATTGTAT	989	51.30	-1.20	-1.331	-1.156	-1.264	-0.913	90.09
543	543 AAATACTGGAGTATTGTATG	687	49.96	-1.20	-1.527	-1.156	-1.386	-0.809	50.4
544	544 AATACTGGAGTATTGTATGG	688	54.30	-1.00	-0.890	-0.982	-0.925	0.688	64.7
545	545 ATACTGGAGTATTGTATGGA	689	57.60	-0.30	-0.406	-0.373	-0.394		76.0
546	546 TACTGGAGTATTGTATGGAT	069	57.60	0.40	-0.406	0.236	-0.162	-0.236	86.0
547	547 ACTGGAGTATTGTATGGATT	691	58.53	1.30	-0.269	1.018	0.220	500'0-	123.4
548	548 CTGGAGTATTGTATGGATTC	692	59.39	2.00	-0.144	1.627	0.529	0.135	121.5
549	549 TGGAGTATTGTATGGATTCT	693	59.39	1.80	-0.144	1.453	0.463	0.210	641.3
550	550 GGAGTATTGTATGGATTCTC	694	96.09	09:0	0.086	0.410		0.286	161.5
551	551 GAGTATTGTATGGATTCTCA	695	59.52	09.0	-0.124	0.410	0.079	0.324	129.9
552	552 AGTATTGTATGGATTCTCAG	969	58.31	1.10	-0.302	0.844	0.134	0.374	88.7
553	553 GTATTGTATGGATTCTCAGG	697	60.87	1.10		0.844			112.5
554	554 TATTGTATGGATTCTCAGGC	869	61.97	1.10	0.236	0.844	0.467	925.0	134.6
555	555 ATTGTATGGATTCTCAGGCC	669	66.52	1.10	0.902	0.844	0.880		191.6
556	556 TIGIATGGATICTCAGGCCC	700	70.34	0.70	1	0.496	1.096		
557	557 TGTATGGATTCTCAGGCCCA	701	71.11	0.20	1.577	0.062	1.001	0.738	332.2
558	558 GTATGGATTCTCAGGCCCAA	702	68.95	00.0	1.259	-0.112	0.738	0.764	415.6
556	559 TATGGATTCTCAGGCCCAAT	703	65.78	00.0	0.795	-0.112	0.450		
560	560 ATGGATTCTCAGGCCCAATT	704	66.68	00.0	0.925	-0.112	0.531	0.737	464.0
561	561 TGGATTCTCAGGCCCAATTT	705	67.04	0.20	0.979	0.062			492.5
295	562 GGATTCTCAGGCCCAATTTT	902	67.51	1.10	1.048	0.844	0.970	0.624	639.
563	563 GATTCTCAGGCCCAATTTTT	707	65.34	1.30	0.729	1.018	0.839	0.595	512.4
564	564 ATTCTCAGGCCCAATTTTTG	708	63.94	09.0	0.524	0.410	0.481	0.513	393.4
565	565 TTCTCAGGCCCAATTTTGA	709	65.24	0.20	0.716	0.062	0.467	0.394	334.3
566	566 TCTCAGGCCCAATTTTGAA	710	62.85	0.20	0.364	0.062	0.249	1870	308.2



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HIV PRT GeneChip™ Data	199.2	164.3	125.6	102.6	91.6	76.2	78.8	84.8			1787.3			Ì	Ì	·									``	~	9036.3
Window- Averaged Composite Score	-0.048	0.223	-0.412	-0.574	889'0-	-0.806	-0.881	-0.841					,	•	0.107	0.293								1.863		1.289	0.945
Composite Score	-0.044	-0.205	-0.518	-0.762	-0.749	-0.855	-0.864	-0.864	-1.027	-1.045	-0.482	-0.145			-0.007	-0.007					2.482					0.961	0.563
AGMFOLD Score	0.062	0.062	-0.547	-0.982	-0.982	-0.982	-0.982	-0.982	-0.982	-0.808	-0.025	-0.025	-0.025	-0.025	-0.025	-0.025		0.931	1.366		4.236		4.236	4.236	0.670	0.062	0.062
Score	-0.109	-0.369	-0.501	-0.627	-0.606	-0.777	-0.792	-0.792	-1.055	-1.190	-0.762	-0.219	0.004	0.004	0.004	0.004	0.212	0.557	1.030	1.351	1.408	1.408	1.588	1.820	1.561	1.513	0.870
∆Gмғо∟р (kcal/mole @ 35 °C)	0.20	0.20	-0.50	-1.00	-1.00	-1.00	-1.00	-1.00	-1.00	-0.80	0.10	0.10	0.10	0.10	0.10	0.10	0.40	1.20	1.70	4.00	5.00	5.00	2.00	2.00	06:0	0.20	0.20
RNA/DNA Tm (°C)	59.65	57.85	56.95	56.09	56.23	55.07	54.96	54.96	53.17	52.25	55.17	58.88	60.39	60.39	60.39	60.39	61.81	64.17	62.39	69.58	96.69	96.69	71.19	72.77	71.01	70.68	66.30
SEQ ID NO:	711	712	713	714	715	716	717	718	719	720	721	722	723	724	725	726	727	728	729	730	731	732	733	734	735	736	737
DNA Probe Sequence	567 CTCAGGCCCAATTTTGAAA	568 TCAGGCCCAATTTTTGAAAT	569 CAGGCCCAATTTTGAAATT	570 AGGCCCAATTTTGAAATTT	571 GGCCCAATTTTTGAAATTTT	572 GCCCAATTTTGAAATTTTC	573 CCCAATTTTGAAATTTTCC	574 CCAATTTTGAAATTTTCCC	575 CAATTTTGAAATTTTCCCT	576 AATTTTGAAATTTTCCCTT	577 ATTTTGAAATTTTCCCTTC	578 TTTTTGAAATTTTCCCTTCC	579 TTTTGAAATTTTCCCTTCCT	580 TITGAAATITICCCTICCTT	581 TIGAAATITICCCTICCTIT	582 TGAAATTTTCCCTTCCTTTT	583 GAAATTTTCCCTTCCTTTTC	584 AAATTTTCCCTTCCTTTTCC	585 AATTITCCCTTCCTTTTCCA	586 ATTTTCCTTTCCAT	587 TTTTCCCTTCCTTTTCCATT	588 TITCCCTICCTITICCATIT	589 TICCCTICCTITICCALITIC	590 recerrectrificative	591 CCCTTCCTTTTCCATTTCTG	592 CCTTCCTTTTCCATTTCTGT	593 CTTCCTTTTCCATTTCTGTA
p5 Probe Position	567	568	569	570	571	572	573	574	575	576	577	578	579	580	581	582	583	584	585	586	283	588	586	265	591	592	593



DNA Probe Sequence	SEQ ID	RNA/DNA T_ (°C)	ΔG _{MFOLD} (kcal/mole	T _m Score	∆GMFOLD Score	Composite Score	Window- Averaged	GeneChip™
		Ē	(© 35 °C)				Composite Score	Data
594 ITCCTTTTCCATTTCTGTAC	738	64.87	0.20	0.660	0.062	0.433		8251.8
595 TCCTTTTCCATTTCTGTACA	739	65.74	0.20	0.788	0.062	0.512	0.257	20788.0
596 CCTTTTCCATTTCTGTACAA	740	62.11	0.20	0.256	0.062	0.182	0.024	7073.9
597 CTTTTCCATTTCTGTACAAA	741	56.39	0.20	-0.583	0.062	-0.338		2932.4
598 TTTTCCATTTCTGTACAAAT	742	54.49	0.20	-0.862	0.062	-0.511	008.0-	1897.3
599 TTTCCATTTCTGTACAAATT	743	54.49	-0.30		-0.373	-0.676		2158.1
600 TICCATITICIGIACAAATIT	744	54.49	-0.30	-0.862	-0.373	-0.676	809'0-	2215.9
601 rccattrcrgracaartrc	745	55.43	-0.30	-0.724	-0.373	-0.591	-0.695	2168.6
602 CCATTTCTGTACAAATTTCT	746	56.07	-0.30	-0.631	-0.373	-0.533		
603 CATTTCTGTACAAATTTCTA	747	51.65	-0.30	-1.278	-0.373	-0.934	0.708	1277.2
604 ATTTCTGTACAAATTTCTAC	748	50.83	-0.10	-1.398	-0.199	-0.943	-0.736	1944.8
605 TTTCTGTACAAATTTCTACT	749	52.78	0.40	-1.112	0.236	-0.600	-0.790	
606 TTCTGTACAAATTTCTACTA	750	51.90	0.40	-1.242	0.236		-0.876	
607 TCTGTACAAATTTCTACTAA	751	49.84	0.40	-1.544	0.236			
608 CTGTACAAATTTCTACTAAT	752	48.73	0.40	-1.707	0.236			
609 TGTACAAATTTCTACTAATG	753	46.88	0.40	-1.979	0.236	-1.137	-0.845	1678.1
610 GTACAAATTTCTACTAATGC	754	50.66	09.0	-1.424	0.410			5877.3
611 TACAAATTTCTACTAATGCT	755	49.82	09.0	-1.547	0.410	-0.803	-0.849	4461.0
612 ACAAATTTCTACTAATGCTT	756	50.65	09.0	-1.425	0.410	-0.728	-0.816	5943.2
613 CAAATTTCTACTAATGCTTT	757	50.46	09.0	-1.453	0.410		-0.753	
614 AAATTTCTACTAATGCTTTT	758	49.47	09.0	-1.599	0.410	-0.836	-0.745	6875.0
615 AATTTCTACTAATGCTTTTA	759	50.61	09.0	-1.431	0.410	-0.731		
616 ATTTCTACTAATGCTTTTAT	760	52.40	0.20	-1.169	0.062			
617 TTTCTACTAATGCTTTTATT	761	52.72	0.20	-1.122	0.062	-0.672		6885.8
618 TTCTACTAATGCTTTTATTT	762	52.72	0.20	1.122	0.062			
619 TCTACTAATGCTTTTATTTT	763	52.72	0.20	1.122	0.062			
620 CTACTAATGCTTTTATTTT	764	51.81	0.20	1.255	0.062	-0.755	0.718	5952.5

ξ	2.8	4.0	8.5	0.1	9.4	3.8	9.7	1.4	1.6	7.2	ე. 1	3.0	4.7	4.1	9.7	7.7	9.7	1.7	5267.4	3922.8	3608.6	1881.6	1658.0	369.8	605.8	933.2	441.8
HIV PRT GeneChip™ Data	2662.8	3034.0	2198.5	1670.1	3039.4	3873.8	3609.	4891.4	3071.6	2667.2	2293.	2123.0			3659.7							,	•				
Window- Averaged Composite Score	-0.724	799'0-	-0.513	-0.315	•	0.177	0.363	0.464	087'0	108'0					0.372		695'0						0.139			/98'0-	0.334
Composite Score	-0.903	-0.741	609.0-	-0.694	-0.294	0.405	0.631		0.763	969'0	0.013	-0.183	-0.218		0.473	0.799	0.877				0.128		-0.018			-0.506	-0.603
∆GMFOLD Score	0.062	0.062	0.062	0.236	0.844	1.714	2.323	2.583	2.583	2.583	1.366	0.931	0.931	0.931	0.931	0.931	0.931	0.757	0.757	-0.634	-1.243	-1.243	-1.243	-1.243	-1.243	-1.243	-1.243
Score	-1.494	-1.233	-1.021	-1.263	-0.992	-0.397	-0.406	0.087	-0.353	-0.461	-0.816	-0.866	-0.922	-0.442	0.192	0.717	0.843	0.787	0.787	0.718	0.968	0.733	0.733	0.433	-0.144	-0.055	0.211
∆Gмғо∟р (kcal/mole @ 35 °C)	0.20	0.20	0.20	0.40	1.10	2.10	2.80	3.10	3.10	3.10	1.70	1.20	1.20	1.20	1.20	1.20	1.20	1.00	1.00	09:0-	-1.30	-1.30	-1.30	-1.30	-1.30	-1.30	-1.30
RNA/DNA T _m (°C)	50.18	51.96	53.41	51.76	53.61	57.66	57.60	96.09	57.96	57.22	54.81	54.46	54.08	57.36	61.67	65.26	66.11	65.73	65.73	65.26	66.97	65.36	65.36	63.32	59.38	59.99	58.93
SEQ ID NO:	765	991	167	768	697	770	771	772	773	774	775	176	777	778	779	780	781	782	783	784	785	786	787	788	789	790	791
DNA Probe Sequence	621 TACTAATGCTTTTATTTTTT	622 ACTAATGCTTTTATTTTTC	623 CTAATGCTTTTATTTTTTT	624 TAATGCTTTTATTTTTTTTT	625 AATGCTTTTATTTTTTTTTC	626 ATGCTTTTATTTTTTTTTT	627 TGCTTTTTTTTTTCTG	628 GCTTTTATTTTTCTTCTGT	629 CTTTTATTTTTCTTCTGTC	630 TTTTATTTTTCTTCTGTCA	631 TTTATTTTTTCTGTCAA	632 TTATTTTTTCTTCTGTCAAT	633 TATTTTTTTTTGTCAATG	634 ATTTTTTTTTTGTCAATGG	635 TTTTTTTTTTGTCAATGGC	636 TTTTTTTTTGTCAATGGCC	637 TTTTCTGTCAATGGCCA	638 TTTCTTCTGTCAATGGCCAT	639 TICTICIGICAAIGGCCATT	640 TCTTCTGTCAATGGCCATTG	641 CTTCTGTCAATGGCCATTGT	642 TTCTGTCAATGGCCATTGTT	643 TCTGTCAATGGCCATTGTTT	644 CTGTCAATGGCCATTGTTTA	645 TGTCAATGGCCATTGTTTAA	646 GTCAATGGCCATTGTTTAAC	647 TCAATGGCCATTGTTTAACT
p5 Probe Position	621	622	623	624	625	626	627	628	629	630	631	632	633	634	635	636	637	638	639	640	641	642	643	644	645	646	647



	SEQ ID	Tm (°C)	∆Gwro∟b (kcal/mole @ 35 °C)	Score	∆GMFOLD Score	Composite Score	Window- Averaged Composite	HIV PRT GeneChip™ Data
648 CAATGCCATTGTTTAACTT	792	57.97	06.0-	-0.352	-0.895	-0.558	Score 0.284	545 6
649 AATGGCCATTGTTTAACTTT	793	57.07			0.670	-0.045		781.4
650 ATGGCCATTGTTTAACTTTT	794	59.31	06.0	-0.156	0.670	0.158		1027.3
651 TGGCCATTGTTTAACTTTTG	795	59.24	06:0	-0.165	0.670	0.152		1102.5
652 GGCCATTGTTTAACTTTTGG	962	61.84	0.30	0.216	0.149			935.7
653 GCCATTGTTTAACTTTTGGG	797	61.84	-0.10	0.216	-0.199	0.058	0.218	403.7
654 CCATTGTTTAACTTTTGGGC	798	61.84	0.30	0.216	0.149	0.190	0.254	269.3
655 CATTGTTTAACTTTTGGGCC	799	61.84	06.0	0.216	0.670	0.389	0.299	296.8
656 ATTGTTTAACTTTTGGGCCA	800	61.84	06.0	0.216	0.670	0.389	798.0	449.4
657 TIGITIAACTITIGGGCCAT	801	61.84	06.0	0.216	0.670	0.389	775.0	448.
658 TGTTTAACTTTTGGGCCATC	802	62.91	06.0	0.373	0.670	0.486	0,340	584.9
659 GTTTAACTTTTGGGCCATCC	803	66.73	0.40	0.934	0.236	0.669	925'0	1032.4
660 TTTAACTTTTGGGCCATCCA	804	64.79	-0.70	0.649	-0.721	0.128	0.235	737.8
661 TTAACTTTTGGGCCATCCAT	805	64.44	-1.20	0.598	-1.156	690.0-	0.274	950.2
662 TAACTTTTGGGCCATCCATT	908	64.44	-1.20	0.598	-1.156	690.0-	0.310	1308.0
663 AACTTTTGGGCCATCCATTC	807	66.42	-1.20	0.888	-1.156	0.111	0.296	2360.1
664 ACTTTTGGGCCATCCATTCC	808	72.21	-1.20	1.738	-1.156	0.638	0.387	4946.0
665 CTTTTGGGCCATCCATTCCT	809	73.53	-1.20	1.930	-1.156	0.758	0.480	6789.2
666 TTTTGGGCCATCCATTCCTG	810	71.49	-1.20	1.632	-1.156	0.573	095'0	8150.6
667 TTTGGGCCATCCATTCCTGG	811	73.62	-1.20	1.945	-1.156	0.766	0.622	7589.0
668 Treseccarccarresc	812	77.43	-2.80	2.504	-2.547	0.584	0.580	13914.0
669 regeccarccarrecreger	813	78.94	-3.50	2.725	-3.156	0.490	005'0	17513.0
670 GGGCCATCCATTCCTGGCTT	814	79.51	-3.50	2.809	-3.156	0.542	0.449	19883.0
671 GGCCATCCATTCCTGGCTTT	815	77.37	-3.50	2.494	-3.156	0.347	0.324	20103.0
672 GCCATCCATTCCTGGCTTTA	816	74.28	-3.10	2.040	-2.808	0.198	0.214	18622.0
673 CCATCCATTCCTGGCTTTAA	817	67.92	-1.30	1.109	-1.243	0.215	0.122	16915.0
674 CATCCATTCCTGGCTTTAAT	818	64.36	-1.30	0.585	-1.243	-0.109	0.028	13910.0



E L		4.0	0.0	0.0	9726.8	9.7	6800.4	5445.6	2901.6	1174.2	521.3	611.1	287.6	109.5	59.5	62.1	59.4	0.89	72.9	62.2	64.5	53.5	57.8	341.0	54.8	47.1	59.7	47.0
HIV PRT	Data	12524.0	11890.0	12839.0																								
Window-	Composite Score	0000	500.0-	070.0	0.126	0.238	0.336	0.396	0.434	0.431	0.268												·					0.425
ၓ	a 1000	-0.185	-0.152	-0.118	060.0	0.223	0.532	0.494			0.301		0.205				-0.745			-0.442			-0.575	-0.210	0.280	0.823		0.586
∆GMFOLD	Score	-1.243	-1.243	-0.895	0.670	1.192	1.540	1.540		1.540	1.540	1.540	1.453	0.236		-0.982	-0.982	1	-0.982	-0.982	İ	-0.982	-0.982	-0.808	-0.025	0.844	0.670	0.496
E	Score	0.464	0.516	0.359	-0.266	-0.371	-0.086	-0.146	0.024	-0.087	-0.458	0.368	0.560	1.130	0.989	0.847	0.599	0.308	0.207	0.111	0 -0.164	0 -0.303	0 -0.326	0 0.157	0 0.467	0.810	1.131	0 0.640
∆GMFOLD.	(kcal/mole @ 35 °C)	-1.30	-1.30	-0.90	06.0	1.50	1.90	1.90	1.90	1.90	1.90	1.90	1.80	0.40	-0.80	-1.00	-1.00	-1.00	-1.00	-1.00	-1.00	-1.00	-1.00	-0.80	0.10	1.10	0.90	3 0.70
RNA/DNA	() E	63.53	63.88	62.81	58.55	57.84	59.78	59.37	60.53	59.77	57.25	57.86	56.55	52.66	53.62	54.59	56.28	58.27	61.78	59.61	59.25	58.30	58.15	61.44	63.55	62.89	68.08	64.73
SEQ ID	.; O	819	820	821	822	823	824	825	826	827	828	829	830	831	832	833	834	835	836	837	838	839	840	841	842	843	844	845
DNA Probe Sequence		675 ATCCATTCCTGGCTTTAATT	676 TCCATTCCTGGCTTTAATTT	677 CCATTCCTGGCTTTAATTTT	678 CATTCCTGGCTTTAATTTTA	679 ATTCCTGGCTTTAATTTTAC	680 ITCCTGGCTTTAATTTTACT	681 TCCTGGCTTTAATTTTACTG	682 CCTGGCTTTAATTTTACTGG	683 CTGGCTTTAATTTTACTGGT	684 TGGCTTTAATTTTACTGGTA	685 GGCTTTAATTTTACTGGTAC	686 GCTTTAATTTTACTGGTACA	687 CTTTAATTTTACTGGTACAG	688 TTTAATTTTACTGGTACAGT	689 TTAATTTTACTGGTACAGTC	690 TAATTTTACTGGTACAGTCT	691 AATTTTACTGGTACAGTCTC	692 ATTTTACTGGTACAGTCTCA	693 TTTTACTGGTACAGTCTCAA	694 TTTACTGGTACAGTCTCAAT	695 TTACTGGTACAGTCTCAATA	696 TACTGGTACAGTCTCAATAG	697 ACTGGTACAGTCTCAATAGG	698 CTGGTACAGTCTCAATAGGG	699 TGGTACAGTCTCAATAGGGC	700 GGTACAGTCTCAATAGGGCT	701 GTACAGTCTCAATAGGGCTA
p5 Probe	Position	675	676	677	678	629	089	681	682	683	684	685	989	687	688	689	069	691	692	693	69	69	969	/69	369	369	707	.02

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HIV PRT GeneChip™ Data	49.3	55.0	49.0	45.	115.6	50.6	48.0	50.5	44.	43.	45.2	47.4	50.0	47.8	50.2								132.5		141.0	282.0	948.6
Window- Averaged Composite Score	0.425	0.388	0.275	0.220	0.189	0.133	0.075	0.028	181'0-	867'0-	-0.432	-0.569	-0.717	-0.867	-1.022	-1.096	-1.088	-1.072	-1.011	-0.924			-0.737		-0.720	629'0-	679'0 -
Composite Score	0.096	0.147	0.091	0.278	0.560	0.167	0.202	-0.124	-0.242	-0.316	-0.442	-0.581		-0.739	-1.080		-1.371						699'0-		-1.025	-0.897	-0.671
∆G _{MFOLD} Score	0.496	0.496	0.496	0.670	0.844	0.236	1.105	1.105	1.453	1.540	0.757	0.323	0.323	0.323	0.323	0.323	0.323	0.323	0.496	1.366	1.453	1.279	0.931	0.931	0.844	0.844	0.844
T _m Score	-0.149	-0.067	-0.158	0.037	0.386	0.125	-0.352	-0.877	-1.281	-1.454	-1.178	-1.135	-1.135	-1.390	-1.940	-2.254	-2.408	-2.655		-1.682	_	-1.838	-1.649	-1.801	-2.171	-1.965	-1.599
∆Gмғо∟о (kcal/mole @ 35°C)	0.70	0.70	0.70	0.90	1.10	0.40	1.40	1.40	1.80	1.90	1.00	0.50	0.50	0.50	09'0	0.50	0.50	0.50	0.70	1.70	1.80	1.60	1.20	1.20	1.10	1.10	1.10
RNA/DNA T _m (°C)	59.35	59.91	59.29	60.62	63.00	61.22	57.97	54.39	51.64	50.45	52.34	52.63	52.63	50.89	47.14	45.00	43.95	42.27	46.18	48.90	47.39	47.84	49.12	48.09	45.57	46.97	49.46
SEQ ID NO:	846	847	848	849	850	851	852	853	854	855	856	857	828	859	860	861	862	863	864	865	998	867	898	698	870	871	872
DNA Probe Sequence	702 TACAGTCTCAATAGGGCTAA	703 ACAGTCTCAATAGGGCTAAT	704 CAGTCTCAATAGGGCTAATG	705 AGTCTCAATAGGGCTAATGG	706 GTCTCAATAGGGCTAATGGG	707 TCTCAATAGGGCTAATGGGA	708 CTCAATAGGGCTAATGGGAA	709 TCAATAGGGCTAATGGGAAA	710 CAATAGGGCTAATGGGAAAA	711 AATAGGGCTAATGGGAAAAT	712 ATAGGGCTAATGGGAAAATT	713 TAGGGCTAATGGGAAAATTT	714 AGGGCTAATGGGAAAATTTA	715 GGGCTAATGGGAAAATTTAA	716 GGCTAATGGGAAAATTTAAA	717 GCTAATGGGAAAATTTAAAG	718 CTAATGGGAAAATTTAAAGT	719 TAATGGGAAAATTTAAAGTG	720 AATGGGAAAATTTAAAGTGC	721 ATGGGAAAATTTAAAGTGCA	722 TGGGAAAATTTAAAGTGCAA	723 GGGAAATTTAAAGTGCAAC	724 GGAAAATTTAAAGTGCAACC	725 GAAAATTTAAAGTGCAACCA	726 AAAATTTAAAGTGCAACCAA	727 AAATTTAAAGTGCAACCAAT	728 AATTTAAAGTGCAACCAATC
p5 Probe Position	702	703	704	702	902	707	708	607	710	711	712	713	714	715	716	717	718	719	720	721	722	723	724	725	726	727	728



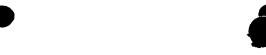
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HIV PRT GeneChip™ Data		1815.1	3188.2	3566.1	2925.1	3233.2	3615.6	3994.8	4033.0	3380.2	4288.7	744.1				-		152.7	270.5	498.7	891.0	Ì		-				715.1
Window- Averaged Composite	Score	795.0	-0.426	797'0'	780.0-	0.014	0.085	0.165			-0.173	-0.445				-1.262	-1.168											-0.445
Composite Score		-0.363	-0.366	-0.317	-0.331	-0.036	0.254	0.550	0.345	0.128	0.242	-0.519	-0.946			-1.840	-1.778		-0.820	•			-0.132		-0.387		-0.663	-0.503
∆GMFOLD Score		0.844	0.844	0.757	0.757	0.757	0.757	0.757	0.757	0.757	0.757	-0.373	-1.504	-2.112	-2.982	-3.417	-3.243	-2.373	-1.764	-0.373	0.062	0.062		0.062	0.062		0.062	0.062
Score		-1.104	-1.109	-0.976	-0.999	-0.522	-0.055	0.422	<u> </u>	-0.257	-0.073	-0.608	-0.605	-0.332	-0.362	-0.874	-0.880	-0.293	-0.241	١.	0.049	0.248	-0.250	0.279	0.661	0.922	1.107	0.849
AGMFOLD (kcal/mole	G S D	1.10	1.10	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	-0.30	-1.60	-2.30	-3.30	-3.80	-3.60	-2.60	-1.90	-0.30	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
RNA/DNA Tm (°C)		52.84	52.81	53.71	53.56	56.81	59.99	63.25	61.00	58.62	59.87	56.22	56.24	58.10	57.90	54.41	54.37	58.37	58.73	58.73	60.70	62.06	58.66	58.47	55.86	54.08	52.82	54.58
SEQ ID NO:		873	874	875	876	877	878	879	880	881	882	883	884	885	988	887	888	889	890	891	892	893	894	895	968	897	868	899
DNA Probe Sequence		729 ATTTAAAGTGCAACCAATCT	730 TTTAAAGTGCAACCAATCTG	731 TTAAAGTGCAACCAATCTGA	732 TAAAGTGCAACCAATCTGAG	733 AAAGTGCAACCAATCTGAGT	734 AAGTGCAACCAATCTGAGTC	735 AGTGCAACCAATCTGAGTCA	736 GTGCAACCAATCTGAGTCAA	737 TGCAACCAATCTGAGTCAAC	738 GCAACCAATCTGAGTCAACA	739 CAACCAATCTGAGTCAACAG	740 AACCAATCTGAGTCAACAGA	741 ACCAATCTGAGTCAACAGAT	742 CCAATCTGAGTCAACAGATT	743 CAATCTGAGTCAACAGATTT	744 AATCTGAGTCAACAGATTTC	745 ATCTGAGTCAACAGATTTCT	746 TCTGAGTCAACAGATTTCTT	747 CTGAGTCAACAGATTTCTTC	748 TGAGTCAACAGATTTCTTCC	749 GAGTCAACAGATTTCTTCCA	750 AGTCAACAGATTTCTTCCAA	751 GTCAACAGATTTCTTCCAAT	752 TCAACAGATTTCTTCCAATT	753 CAACAGATTTCTTCCAATTA	754 AACAGATTTCTTCCAATTAT	755 ACAGATTTCTTCCAATTATG
p5 Probe Position		729	730	731	732	733	734	735	736	737	738	739	740	741	742	743	744	745	746	747	748	749	750	751	752	753	754	755



. ¥	8.8	7.7	9.0	3.7	9.0	4.7	3.3	5.3	0.0	5.4	3.4	1	5.7	1	-	4.7	ω ω	0.0	5.4	838.8	3.1	7.0	872.0	897.6	2.2	5.6	5.9
HIV PRT GeneChip™ Data	833.	1067.	1225.	1028	1419.0	1437.4	1518.3	1560.3	1100.0	1096.4	1103.4	738.	596.7	548.	701.1	724.	1129.8	1214.0	1425.4	83	1173.	1367.	87.	.68	962.	1382.6	1132.9
Window- Averaged Composite Score	-0.460	-0.445	0.40	-0.382	-0.378	-0.348	-0.325	-0.262	-0.244	-0.216	-0,124	-0.005		0.161	0.274	0.420	905.0		07970	0.499	0.427		0.192	0.100	-0.024	060'0-	660'0-
Composite Score	-0.284	-0.372	-0.359	-0.491	-0.442	-0.356	-0.369	-0.259	-0.161	-0.198	-0.047	-0.319	-0.159	0.272	0.575	0.256	0.551	0.745	0.703	0.438			0.108	0.053	-0.115	-0.083	-0.143
∆GMFOLD Score	0.062	0.062	0.149	-0.199	-0.199	-0.199	-0.199	-0.199	-0.199	-0.199	-0.199	0.149	0.844	0.844	0.844	l	-0.634	-0.634	-0.634	-0.634	-0.895		-0.895	-0.895	-0.895	-0.895	-0.895
T _m Score	-0.496	-0.638	-0.670	-0.670	-0.591	-0.452	-0.474	-0.295	-0.138	-0.198	0.046	-0.605	-0.774	-0.079	0.409	L	1.278	1.591	1.523	1.095	1.343	1.275	0.723	0.634	0.362	0.414	0.317
∆G _{MFOLD} (kcal/mole @ 35 °C)	0.20	0.20	0.30	-0.10	-0.10	-0.10	-0.10	-0.10	-0.10	-0.10	-0.10	0.30	1.10	1.10	1.10	-0.20	09:0-	-0.60	09'0-	09.0-	06.0-	06.0-	06:0-	06'0-	06.0-	06'0-	06'0-
RNA/DNA Tm (°C)	56.99	56.02	55.80	55.80	56.34	57.29	57.14	58.36	59.43	59.02	89.09	56.24	55.09	59.83	63.16	64.38	80.69	71.21	70.75	67.83	69.52	90.69	65.30	64.69	62.84	63.19	62.53
SEQ ID NO:	006	901	902	903	904	905	906	907	806	606	910	911	912	913	914	915	916	917	918	919	920	921	922	923	924	925	926
DNA Probe Sequence	756 CAGATTTCTTCCAATTATGT	757 AGATTTCTTCCAATTATGTT	758 GATTTCTTCCAATTATGTTG	759 ATTTCTTCCAATTATGTTGA	760 TITCTICCAATIAIGITGAC	761 TTCTTCCAATTATGTTGACA	762 TCTTCCAATTATGTTGACAG	763 CTTCCAATTATGTTGACAGG	764 TTCCAATTATGTTGACAGGT	765 TCCAATTATGTTGACAGGTG	766 CCAATTATGTTGACAGGTGT	767 CAATTATGTTGACAGGTGTA	768 AATTATGTTGACAGGTGTAG	769 ATTATGTTGACAGGTGTAGG	770 TTATGTTGACAGGTGTAGGT	771 TATGTTGACAGGTGTAGGTC	772 ATGTTGACAGGTGTAGGTCC	773 TGTTGACAGGTGTAGGTCCT	774 GTTGACAGGTGTAGGTCCTA	775 TTGACAGGTGTAGGTCCTAC	776 TGACAGGTGTAGGTCCTACT	777 GACAGGTGTAGGTCCTACTA	778 ACAGGTGTAGGTCCTACTAA	779 САССТВСТВСТВАТ	780 AGGTGTAGGTCCTACTAATA	781 GGTGTAGGTCCTACTAATAC	782 GTGTAGGTCCTACTAATACT
p5 Probe Position	756	757	758	759	760	761	762	763	764	765	766	797	768	169	770	771	772	773	774	775	776	777	778	779	780	781	782



DNA Probe Sequence	SEQ ID	RNA/DNA	AGMEDI D	E	∆GMFOLD	ၓ	-MODUIA	באיז אור
	Ö	T _m (°C)	(kcal/mole @ 35 °C)	Ð	Score	Score	Averaged Composite Score	GeneChip™ Data
783 TGTAGGTCCTACTAATACTG	927	59.27	-0.90	-0.160	-0.895	-0.439		
784 GTAGGTCCTACTAATACTGT	928	62.53	-0.50	0.317	-0.547	-0.011	070'0-	1932.9
785 TAGGTCCTACTAATACTGTA	929	58.77	0.70	-0.234	0.496	0.043		1634.4
786 AGGTCCTACTAATACTGTAC	930	59.91	0.50	-0.067	0.323	0.081		
787 GGTCCTACTAATACTGTACC	931	63.54	0.50	0.466	0.323	0.411	0.116	
788 GTCCTACTAATACTGTACCT	932	62.91	0.50	0.373				
789 TCCTACTAATACTGTACCTA	933	59.31	0.50	-0.155	0.323	0.026		
790 CCTACTAATACTGTACCTAT	934	57.99	0.50	-0.348	0.323	-0.093		
791 CTACTAATACTGTACCTATA	935	53.68	0.50	-0.981	0.323			1
792 TACTAATACTGTACCTATAG	936	51.92	0.70	-1.240	0.496	-0.580		
793 ACTAATACTGTACCTATAGC	937	56.45	1.20	-0.574	0.931	·		
794 CTAATACTGTACCTATAGCT	938	57.85	1.20	-0.369	0.931			
795 TAATACTGTACCTATAGCTT	939	56.25	1.20	-0.604	0.931	-0.021	•	
796 AATACTGTACCTATAGCTTT	940	57.14	1.20	0.473	0.931			
797 ATACTGTACCTATAGCTTTA	941	58.55	1.20	0.266	0.931			
798 TACTGTACCTATAGCTTTAT	942	58.55	1.20	0.266	0.931			
799 ACTGTACCTATAGCTTTATG	943	59.06	1.20	0.192	0.931			
800 CTGTACCTATAGCTTTATGT	944	61.64	1.30	0.187	1.018	0.503		
801 TGTACCTATAGCTTTATGTC	945	61.08	1.10	0.105	0.844			
802 GTACCTATAGCTTTATGTCC	946	65.16	1.10	0.703	0.844			
803 TACCTATAGCTTTATGTCCA	947	63.16	1.10	0.409	0.844			
804 ACCTATAGCTTTATGTCCAC	948	64.30	1.30	0.577	1.018			
805 CCTATAGCTTTATGTCCACA	949	64.94	1.30	0.671	1.018	0.803		
806 CTATAGCTTTATGTCCACAG	950	61.34	1.10	0.143	0.844	0.409	9 0.484	
807 TATAGCTTTATGTCCACAGA	951	60.70	1.10	0.048	0.844			
808 ATAGCTTTATGTCCACAGAT	952	61.27	09.0	0 0.132	0.410	0.238	9 0.414	
900 TAGGGGTTTATTATTATT	953	61.63	3 0.60	0.186	0.410	0.271	1 0.337	4232.6



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HIV PRT GeneChip™ Data	5252.8	6823.9	4829.8	4333.7	3801.0	3528.2	2080.0	913.8	1228.3	238.1	219.4	138.6	112.7	133.8	296.8	279.7	484.4	502.0	637.3	489.0	808.7	903.2	1709.3	2103.9	3973.4	6462.3	9749.0
Window- Averaged Composite Score	0.283	0.232	0.193	0.173	444.0	0.142	0.129	0.145	0.122	790.0	-0.078	-0.169	-0.273	-0.327	-0.387	0.442	-0.498	-0.510	0.569	759.0	0.712	-0.738	202'0-	-0.601	-0.468	-0.330	-0.167
Composite Score	0.356	0.472	0.265	0.029	-0.004	-0.041	0.134	0.153	0.457	0.173	0.144	-0.162	-0.430	-0.883	-0.485	-0.270	-0.206	-0.277	-0.541	-0.826		-0.899	-0.883	-0.597	-0.455	-0.326	-0.081
∆G _{MFOLD} Score	0.410	0.410	0.410	0.410	0.410	0.410	0.410	0.410	0.410	0.583	0.844	0.670	-0.199	-1.243	-0.199	0.410	0.583	0.062	-0.547	-1.069		-1.069	-1.156	-1.156	-1.156	-0.982	-0.373
Score	0.324	0.511	0.176	-0.205	-0.257	-0.318	-0.036	-0.004	0.486	-0.078	-0.285	-0.673	-0.571	-0.663	-0.659	1	-0.689	-0.485	-0.538	-0.678	-0.794	-0.794	-0.715	l	-0.025	0.076	0.097
∆G _{MFOLD} (kcal/mole @ 35 °C)	09.0	09.0	09.0	09'0	09.0	09.0	09.0	09.0	09.0	08.0	1.10	06.0	-0.10	-1.30	-0.10	09.0	08.0	0.20	-0.50	-1.10	-1.30	-1.10	-1.20	-1.20	-1.20	-1.00	-0.30
RNA/DNA Tm (°C)	62.57	63.85	61.56	58.97	58.62	58.20	60.12	60.34	63.68	59.83	58.43	55.78	56.48	55.85	55.87	55.69	25.67	90'.29	56.70	52.75	54.95	54.95	55.49	58.64	60.20	98.09	61.03
SEQ ID NO:	954	955	926	957	958	959	096	961	962	963	964	965	996	196	896	696	970	971	972	973	974	975	926	116	978	616	086
DNA Probe Sequence	810 AGCTTTATGTCCACAGATTT	811 GCTTTATGTCCACAGATTTC	812 CTTTATGTCCACAGATTTCT	813 TTTATGTCCACAGATTTCTA	814 TTATGTCCACAGATTTCTAT	815 TATGTCCACAGATTTCTATG	816 ATGTCCACAGATTTCTATGA	817 TGTCCACAGATTTCTATGAG	818 GTCCACAGATTTCTATGAGT	819 TCCACAGATTTCTATGAGTA	820 CCACAGATTTCTATGAGTAT	821 CACAGATTTCTATGAGTATC	822 ACAGATTTCTATGAGTATCT	823 CAGATTTCTATGAGTATCTG	824 AGATTTCTATGAGTATCTGA	825 GATTTCTATGAGTATCTGAT	826 ATTTCTATGAGTATCTGATC	827 TTTCTATGAGTATCTGATCA	828 TTCTATGAGTATCTGATCAT	829 ТСТАТGAGTATCTGATCATA	830 CTATGAGTATCTGATCATAC	831 TATGAGTATCTGATCATACT	832 ATGAGTATCTGATCATACTG	833 TGAGTATCTGATCATACTGT	834 GAGTATCTGATCATACTGTC	835 AGTATCTGATCATACTGTCT	836 статстватсатастетст
p5 Probe Position	810	811	812	813	814	815	816	817	818	819	820	821	822	823	824	825	826	827	828	829	830	831	832	833	834	835	836



T.T.	· "	7817.2	9683.1	8089.0	8.9698	6880.5	7033.7	5406.5	4239.4	3727.4	2665.5	1817.8	1335.9	1526.2	822.7	1227.4	503.0	1174.3	885.5	650.6	615.4	563.4	420.9	536.6	1417.8	4351.2	
HIV PRT GeneChip™	Data	78			8											1		l									
Window-	Composite Score	0.050	200'0	0.055	0.06 7		070'0	280'0-	-0.215	0.378	0.550	969'0-	-0.809	-0.916		996'0-	-0.946		-0.901						•		
Composite Score		-0.037	0.070	0.259	0.159	0.006	0.006	0.006	-0.060	-0.238	-0.533		-1.131	-1.197	-1.019	-0.849	-0.991			-0.988		656'0-		-0.362	-0.079	0.359	
∆GMFOLD Score		0.670	0.670	0.670	0.670	0.670	0.670	0.670	0.496	0.496	0.496	0.496	-0.373	-0.547		-0.547	-0.547	-0.547	-0.547	-0.547	-0.547	-0.547	-0.373	0.410		0.757	
Tm		-0.470	-0.298	0.008	-0.154	-0.401	-0.401	-0.401	-0.401	-0.688	-1.163	-1.426	-1.595	-1.595	-1.309	-1.034	-1.264	-0.894	-0.978	-1.259	-1.185	-1.212	ı	-0.834	-0.378	0.114	
∆GMFOLD (kcal/mole	(© 32 °C)	06.0	06.0	06.0	06.0	06.0	06.0	06.0	0.70	0.70	0.70	0.70	-0.30	-0.50	-0.50	-0.50	-0.50	-0.50	-0.50	-0.50	-0.50	-0.50	•	09.0	09:0	1.00	
RNA/DNA		57.16	58.34	60.42	59.32	57.63	57.63	57.63	57.63	55.68	52.44	50.65	49.49	49.49	51.45	53.32	51.75	54.28	53.70	51.79	52.29	52.11	51.46	54.68	57.79	61.15	
SEQ ID		981	982	983	984	985	986	987	886	686	066	991	992	993	994	995	966	997	866	666	1000	1001	1002	1003	1004	1005	
DNA Probe Sequence		837 TATCTGATCATACTGTCTTA	838 ATCTGATCATACTGTCTTAC	839 TCTGATCATACTGTCTTACT	840 CTGATCATACTGTCTTACTT	841 TGATCATACTGTCTTACTTT	842 GATCATACTGTCTTACTTTG	843 ATCATACTGTCTTACTTTGA	844 TCATACTGTCTTACTTTGAT	845 CATACTGTCTTACTTTGATA	846 ATACTGTCTTACTTTGATAA	847 TACTGTCTTACTTTGATAAA	848 ACTGTCTTACTTTGATAAAA	849 CTGTCTTACTTTGATAAAC	850 TGTCTTACTTTGATAAAACC	851 GTCTTACTTTGATAAAACCT	852 TCTTACTTTGATAAAACCTC	853 CTTACTTTGATAAAACCTCC	854 TTACTTTGATAAAACCTCCA	855 TACTTTGATAAAACCTCCAA	856 ACTTTGATAAAACCTCCAAT	857 CTTTGATAAAACCTCCAATT	858 TTTGATAAAACCTCCAATTC	859 TTGATAAACCTCCAATTCC	860 TGATAAAACCTCCAATTCCC	861 GATAAAACCTCCAATTCCCC	
p5 Probe		837	838	839	840	841	842	843	844	845	846	847	848	849	850	851	852	853	854	855	856	857	828	829	860	861	



Docition	DNA Probe Sequence	SEQ ID	RNA/DNA	∆GMFOLD (Kral/mole	Score	Score	Composite	Window- Averaged	GeneChip™
			Ē	@ 35°C)				Composite Score	Data
864	864 AAAACCTCCAATTCCCCCTA	1008	64.88	1.90	0.663	1.540	0.996	1.074	14811.0
865	865 AAACCTCCAATTCCCCCTAT	1009	66.73	1.90	0.933	1.540	1.164	1.261	15751.0
998	866 AACCTCCAATTCCCCCTATC	1010	70.07	1.80	1.424	1.453	1.435		19661.0
867	867 ACCTCCAATTCCCCCTATCA	1011	73.21	1.80	1.883	1.453	1.720		
868	868 CCTCCAATTCCCCCTATCAT	1012	72.64	1.80	1.801	1.453	1.669	1.327	19376.0
998	869 CTCCAATTCCCCTATCATT	1013	99.69	1.60	1.364	1.279	1.332	1.254	17642.0
870	870 TCCAATTCCCCCTATCATTT	1014	68.21	1.10	1.150	0.844	1.034		
871	871 CCAATTCCCCCTATCATTTT	1015	67.12	1.10	0.991	0.844	0.935	0.931	12669.0
872	872 CAATTCCCCCTATCATTTT	1016	64.02	1.10	ŀ		0.653		
873	873 AATTCCCCCTATCATTTTG	1017	62.80	0.40	0.357	0.236	0.311	0.753	8929.1
874	874 ATTCCCCCTATCATTTTGG	1018	67.28	00.00	1.014	-0.112	0.586	0.715	6148.2
878	875 TTCCCCCTATCATTTTGGT	1019	70.46	00.0	1.480	-0.112			
876	876 TCCCCCTATCATTTTGGTT	1020	70.46	00.0	1.480	-0.112	0.875		
87.	877 CCCCCTATCATTTTGGTTT	1021	69.27	00.00	1.307	-0.112			
878	878 CCCCTATCATTTTGGTTTC	1022	67.18	00.00	1.000	-0.112			
878	879 CCCTATCATTTTGGTTTCC	1023	67.18	00'0	1.000	-0.112	0.577		
88(880 CCTATCATTTTGGTTTCCA	1024	64.63	00.0					
88	881 CTATCATTTTGGTTTCCAT	1025	60.77	-0.50	0.059	-0.547	-0.171		
88,	882 TATCATTTTTGGTTTCCATC	1026	60.20	-0.50	-0.025	-0.547	-0.223		3249.9
88	883 ATCATTTTGGTTTCCATCT	1027	62.83	-0.50	0.361	-0.547			
88	884 TCATTTTTGGTTTCCATCTT	1028	63.21	-0.50	0.416	-0.547	0.050	170'0	5290.0
88	885 CATTTTTGGTTTCCATCTTC	1029	63.21	-0.50	0.416	-0.547	0.050		7451.0
88	886 ATTTTTGGTTTCCATCTTCC	1030	65.88	-0.50	0.809	-0.547	0.293		
88	887 TTTTTGGTTTCCATCTTCCT	1031	67.93	-0.50	1.109	-0.547	0.480	996.0	13722.0
88	888 TTTTGGTTTCCATCTTCCTG	1032	67.42	-0.50	1.035	-0.547			
88	889 TTTGGTTTCCATCTTCCTGG	1033	69.71	06.0-	1.370	-0.895	0.509		10869.0
89	890 TTGGTTTCCATCTTCCTGGC	1034	73.74	-1.30	1.962	-1.243	0.744	9 89'0	16035.0



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HIV PRT GeneChip™ Data	16304.0	14885.0	11910.0	11929.0	11517.0	11822.0	11710.0	7635.3	8378.2	6321.4	7659.0	11621.0	3389.0	3870.6	1992.7	698.3	718.3	372.3	180.6	430.0	904.3	1663.5	2694.2	3222.9	3142.8	5867.0	6641.4
Window- Averaged Composite Score	0.457	0.406	0.358	0.290	0.252	0.237	0.285	798-0	0.400	0.446	0.468	0.420	0.363	0.273	0.160	-0.015	-0.204	-0.339	0.430	-0.455	0.484	-0.510	0.450	0.364	0.310	0.270	0.253
AGMFOLD Composite Score Score	0.812	0.605	0.165	-0.067	0.071	0.174	0.274	0.544	0.501	0.500	0.434	0.434	0.434	0.434	0.268	0.040	-0.130		-0.793	-0.868		-0.366	-0.138	-0.377	-0.495	-0.436	-0.179
∆GMFOLD Score	-1.243	-1.243	-1.243	-1.243	-1.243	-1.243	-0.895	0.670	0.757	0.670	0.496	0.496	0.496	0.496	0.496	0.496	0.496	0.496	0.410	0.410	0.410	0.410	0.410	0.410	0.410	0.583	0.931
T _m Score	2.071	1.737	1.027	0.653	0.877	1.042	0.991	0.466	0.343		0.395	0.395		0.395	0.127	-0.239			-1.530	-1.651		-0.842	-0.474	-0.859	-1.049	-1.061	-0.859
∆G _{MFOLD} (kcal/mole @ 35 °C)	-1.30	-1.30	-1.30	-1.30	-1.30	-1.30	-0.90	0.90	1.00	06.0	0.70	0.70	0.70	0.70	0.70	0.70	0.70		09'0	09.0	09.0	09.0	09.0	09.0	09.0	08.0	1.20
RNA/DNA Tm (°C)	74.48	72.21	67.37	64.82	66.34	67.47	67.12	63.55	62.71	63.06	63.06	63.06	90.69	90.69	61.24	58.74	56.86	54.36	49.93	49.11	52.79	54.63	57.14	54.51	53.21	53.13	54.51
SEQ ID NO:	1035	1036	1037	1038	1039	1040	1041	1042	1043	1044	1045	1046	1047	1048	1049	1050	1051	1052	1053	1054	1055	1056	1057	1058	1059	1060	1061
p5 Probe DNA Probe Sequence	891 TGGTTTCCATCTTCCTGGCA	892 GGTTTCCATCTTCCTGGCAA	893 GTTTCCATCTTCCTGGCAAA	894 TTTCCATCTTCCTGGCAAAC	895 ITCCATCTTCCTGGCAAACT	896 TCCATCTTCCTGGCAAACTC	897 CCATCTTCCTGGCAAACTCA	898 CATCTTCCTGGCAAACTCAT	899 ATCTTCCTGGCAAACTCATT	900 retrectedeaaacteattr	901 CTTCCTGGCAACTCATTTC	902 TTCCTGGCAAACTCATTTCT	903 recrescadacteatrictr	904 CCTGGCAAACTCATTTCTTC	905 CTGGCAAACTCATTTCTTCT	906 TGGCAAACTCATTTCTTA	907 GGCAAACTCATTTCTTAA	908 GCAAACTCATTTCTTCTAAT	909 CAAACTCATTTCTTCTAATA	910 AAACTCATTTCTTAATAC	911 AACTCATTTCTTCTAATACT	912 ACTCATTTCTTCTAATACTG	913 CTCATTTCTTCTAATACTGT	914 TCATTTCTTCTAATACTGTA	915 CATTTCTTCTAATACTGTAT		917 TTTCTTCTAATACTGTATCA
p5 Probe Position																											



		10	- To-	-	I ~	<u></u>	<u></u>	T-		T	_	_	-				_	_	_	ī <u>~</u>			_	T~~	_	<u> </u>	_	100
HIV PRT GeneChip™ Data		7151.9	8134.9	8551.4	5741.7	8575.9	8980.3	10762.0	17037.0	20970.0	23084.0	24474.0	22217.0	19829.0	23548.0	21759.0	22711.0	18134.0	17772.0	17134.0	10969.0	9556.3	3739.9	4088.3	2263.0	1018.0	1319.1	2347.8
Window- Averaged	Score	0.229	0.139	-0.048	0.003	7070	0.248	0.384	0.566	0.682	0.763	0.875	0.910	0.890	0.842	0.773	0.725	902'0	0.611	0.502	0.389	0.336	0.264	0.187	0.106	080'0	0.094	070'0
Composite Score		-0.176	-0.086	-0.023	-0.211	0.140	0.197	0.140	0.553	0.938	0.929	1.065	0.953	0.763	0.927	0.799	0.799	0.589	0.582	0.614	0.635	0.262	0.034	0.003	0.224	0.074	0.074	0.074
∆GMFOLD Score		1.018	1.018	1.018	1.018	1.018	1.018	1.018	1.018	1.018	1.018	0.410	0.410	0.410	0.410	0.410	0.410	0.410	0.757	1.279	1.279	0.323	0.149	-0.982	0.149	0.149	0.149	0.149
T _m Score		-0.908	-0.762	-0.661	-0.964	-0.398	-0.307	-0.398	0.268	0.889	0.874	1.466	1.286	0.979	1.244	1.037	L .	0.699	0.475	0.207	0.241	0.225	-0.036	0.607	0.271	0.028	0.028	0.028
∆GMFOLD (kcal/mole) 3	1.30	1.30	1.30	1.30	1.30	1.30	1.30	1.30	1.30	1.30	09.0	09.0	09.0	09.0	09.0	09.0	09.0	1.00	1.60	1.60	09.0	0.30	-1.00	0.30	0.30	0.30	0.30
RNA/DNA Tm (°C)		54.17	55.17	55.86	53.80	57.65	58.28	59.75	62.19	66.43	66.32	70.36	69.13	67.04	68.85	67.44	67.44	65.13	63.60	61.77	62.01	61.90	60.12	64.50	62.21	99.09	60.56	60.56
SEQ ID NO:		1062	1063	1064	1065	1066	1067	1068	1069	1070	1071	1072	1073	1074	1075	1076	1077	1078	1079	1080	1081	1082	1083	1084	1085	1086	1087	1088
DNA Probe Sequence		918 TICTICIAAIACIGIAICAI	919 TCTTCTAATACTGTATCATC	920 CTTCTAATACTGTATCATCT	921 TICIAAIACIGIAICAICIG	922 TCTAATACTGTATCATCTGC	923 CTAATACTGTATCATCTGCT	924 TAATACTGTATCATCTGCTC	925 AATACTGTATCATCTGCTCC	926 ATACTGTATCATCTGCTCCT	927 TACTGTATCATCTGCTCCTG	928 ACTGTATCATCTGCTCCTGT	929 CTGTATCATCTGCTCTGTA	930 rerarcarcrecrerar	931 GTATCATCTGCTCCTGTATC	932 TATCATCTGCTCCTGTATCT	933 атсатстестсствтатста	934 rearcrecreratera	935 сатстестстетатстват	936 ATCTGCTCCTGTATCTAATA	937 refecteeraratag	938 CTGCTCCTGTATCTAATAGA	939 тестсстетатстатавав	940 GCTCCTGTATCTAATAGAGC	941 CTCCTGTATCTAATAGAGCT	942 recrementable 1	943 ccrerarcraaragaecrre	944 CTGTATCTAATAGAGCTTCC
p5 Probe Position		918	919	920	921	825	923	924	925	926	927	928	676	026	931	832	623	934	932	936	286	826	626	940	941	945	943	944



Marcal Composite Composi		DNA Probe Sequence	SEQ ID	RNA/DNA	∆GMFOLD	E	∆GMFOLD	Composite	Window-	HIV PRT
GECTICCT 1089 60.56 0.30 0.028 0.149 0.074 0.046 SCTTCCTT 1090 61.00 0.30 0.092 0.149 0.141 0.036 TTCCTTTA 1091 58.20 0.30 0.038 0.149 0.141 0.036 TTCCTTTA 1092 58.20 0.30 0.038 0.149 0.0141 0.045 CCTTTAGT 1093 58.39 0.30 0.289 0.149 0.0141 0.045 CTTAGTTG 1094 60.12 0.00 0.036 0.112 0.065 0.045 CTTAGTTG 1095 58.46 0.30 0.289 0.149 0.017 0.045 CTTAGTTG 1096 58.97 0.30 0.758 0.149 0.075 0.044 CTTAGTTG 1096 58.97 0.30 1.340 0.149 0.075 0.044 CTTAGTTG 1096 58.97 0.30 1.340 0.149 0.017 0.045			<u> </u>	<u> </u>	(KCal/mole (@ 35 °C)	9000	Score		Composite Score	Data
SCTTCCTT 1090 61.00 0.30 0.092 0.149 0.114 -0.049 TTCCTTT 1091 58.20 0.30 -0.318 0.149 -0.141 -0.096 TTCCTTTA 1092 58.20 0.30 -0.318 0.149 -0.141 -0.056 TTCCTTTA 1092 58.20 0.30 -0.289 0.149 -0.141 -0.056 CCTTTAGT 1094 60.12 0.00 -0.036 -0.112 -0.065 -0.049 CTTAGTTC 1095 58.46 0.03 -0.280 0.149 -0.177 0.128 CTTAGTTCC 1099 73.89 0.30 1.789 0.149 1.584 1.454 NATTGCC 1099 77.20 0.30 2.789 0.149 1.584 1.454 NATTGCCC 1101 77.20 0.30 2.789 0.149 1.584 1.454 NATTGCCC 1101 76.38 0.30 2.789 0.149 1.584 1.454	1G		1089	99.09		1	0.149	0.074	0.018	
TTTCCTTT 1091 58.20 0.30 -0.318 0.149 -0.141 -0.036 CTTTCCTTTA 1092 58.20 0.30 -0.318 0.149 -0.141 -0.052 CTTTAGT 1093 58.39 0.30 -0.289 0.149 -0.123 -0.078 CTTTAGT 1094 60.12 0.00 -0.036 -0.112 -0.056 -0.149 -0.117 0.128 CTTTAGTT 1095 58.46 0.30 -0.280 0.149 -0.117 0.149 0.332 CTTTAGTT 1095 58.46 0.30 0.280 0.149 0.526 0.526 0.526 0.526 0.526 0.149 0.526 0.526 0.526 0.526 0.149 0.526 0.526 0.526 0.526 0.149 0.526 0.526 0.526 0.526 0.526 0.526 0.526 0.526 0.526 0.526 0.526 0.526 0.526 0.526 0.526 0.526 0.526 0.526 0.526 0.526 0.526 0.526 0.526 0.526 0.499 0.526 0.526 0.526 0.499 0.526 0.526 0.499 0.526 0.499 0.526 0.526 0.490 0.526 0.490 0.526 0.526 0.490 0.526 0.526 0.490 0.526 0.490 0.526 0.490 0.526 0.490 0.526 0.490 0.526 0.490 0.526 0.490 0.526 0.490 0.490 0.526 0.490 0.526 0.490 0.526 0.490 0.526 0.490 0.526 0.490 0.526 0.490 0.526 0.490 0.526 0.490 0.526 0.490 0.526 0.490 0.526 0.490 0.526 0.490 0.526 0.490 0.526 0.490 0.526 0.490 0.526 0.490 0.526 0.490 0.526 0.490 0.526 0.490 0.526 0.490 0.526 0.490 0.526 0.490 0.526 0.490 0.526 0.490 0.526 0.490 0.526 0.490 0.526 0.490 0.526 0.490 0.526 0.490 0.526 0.490 0.526 0.490 0.526 0.526 0.490 0.526 0.490 0.526 0.490 0.526 0.490 0.526 0.490 0.526 0.490 0.526 0.490 0.526 0.490 0.526 0.490 0.526 0.490 0.526 0.490 0.526 0.490 0.526 0.490 0.526 0.490 0.526 0.490 0.526 0.490 0.526 0.490 0.526 0.490 0.526 0.490 0.526 0.490 0.526 0.490 0.526 0.490 0.526 0.490 0.526 0.490 0.526 0.490 0.526 0.490 0.526 0.490 0.526 0.490 0.526 0.490 0.526 0.490 0.526 0.490 0.526 0.490 0.526 0.490 0.526 0.490 0.526 0.490 0.526 0.490 0.526 0.490 0.526 0.490 0.526 0.490 0.526 0.490 0.526 0.490 0.526 0.490 0.526 0.490 0.526 0.490 0.526 0.490 0.526 0.490 0.526 0.490 0.526 0.526 0.490 0.526 0.526 0.490 0.526 0.526 0.490 0.526 0.526 0.490 0.526 0.490 0.526 0.526 0.490 0.526 0.526 0.490 0.526 0.526 0.490 0.526 0.526 0.490 0.526 0.526 0.490 0.526 0.526 0.490 0.526 0.526 0.490 0.526 0.526 0.490 0.526 0.526 0.490 0.526 0.526 0.490 0.526 0.526 0.526 0.526 0.526 0.526 0.526 0.526 0.526 0.526 0.526 0.	GT	ATCTAATAGAGCTTCCTT	1090	61.00			0.149			
TTCCTTTA 1092 58.20 0.30 0.318 0.149 -0.141 -0.057	¥.	CTAATAGAGCTTCCTTT	1091	58.20			0.149			
CCCTTAG 1093 58.39 0.30 -0.289 0.149 -0.123 -0.049 CCTTAGT 1094 60.12 0.00 -0.036 -0.112 -0.065 -0.049 CTTAGT 1095 58.46 0.30 -0.280 0.149 -0.177 0.428 CTTAGT 1096 58.97 0.30 -0.265 0.149 -0.070 0.332 CTAGTAGCC 1097 65.53 0.30 0.758 0.149 0.526 0.526 CTAGTAGCC 1099 69.50 0.30 1.340 0.149 1.286 1.157 CTAGTAGCC 1099 77.20 0.30 2.470 0.149 1.58 1.454 CAGTAGCCC 1101 77.20 0.30 2.789 0.149 1.785 1.650 STAGCCCCC 1101 77.27 0.60 2.889 0.583 2.013 1.765 1.765 SCCCCCTAT 1104 77.27 0.60 2.480 0.410 1.491	ATC	TAATAGAGCTTCCTTTA	1092	58.20		<u></u>	0.149			
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	ğ		1115	73.39			1.105			



DNA Probe Sequence	SEQ ID NO:	RNA/DNA Tm (°C)	∆G _{MFOLD} (kcal/mole	T _m Score	∆GMFoLD Score	Composite Score	Window- Averaged	HIV PRT GeneChip™
			(G 35°C)				Composite Score	Data
ı	1116	70.61	1.40	1.502	1.105	1.351	1.165	6198.4
	1117	67.66	1.20	1.070	0.931	1.017	0.999	
	1118	64.37	1.20	0.587	0.931	0.718		850.
	1119	62.05	1.20	0.248	0.931	0.507	0.570	
	1120	58.56	1.20	-0.265	0.931	0.190	0.436	166.6
	1121	57.28	1.20	-0.452	0.931	0.073	975.0	126.9
	1122	57.91	1.20	-0.361	0.931	0.130	0.279	
_	1123	61.03	1.20	0.097	0.931	0.414	0.173	97.9
	1124	64.18	06.0	0.559	0.670	0.601	280'0	122.3
	1125	64.18	-0.80	0.559	-0.808	0.039	0.013	
	1126	62.63	-1.20	0.332	-1.156	-0.233	£20'0-	396.0
	1127	65.37	-2.30	0.734	-2.112	-0.348	-0.145	
	1128	65.37	-2.80	0.734	-2.547	-0.513		
	1129	65.82	-2.80	0.800	-2.547	-0.472	-0.163	864.5
	1130	70.01	-2.80	1.414	-2.547	-0.091	0.156	1465.7
	1131	73.21	-2.80	1.884	-2.547	0.200	-0.157	2836.9
	1132	74.44	-2.80	2.065	-2.547	0.312	0.137	3589.7
	1133	69.05	-2.80	1.274	-2.547	-0.178	0.058	2100.4
	1134	67.10		0.988	-2.547	-0.355	0.042	1948.7
	1135	66.13	-2.60	0.845	-2.373	-0.378	0.125	1384.3
	1136	66.81	-1.40	0.945	-1.330	0.081	0.187	1192.0
	1137	66.84	0.20	0.950	0.062	0.612	0.304	1221.0
	1138	68.70	0.20	1.223	0.062	0.782	0.427	953.2
_	1139	68.32	0.20	1.167	0.062	0.747	0.515	988.6
	1140	67.11	0.20	0.989	0.062	0.636	0.476	937.8
	1141	64.59	0.50	0.620	0.323	0.507	0.333	852.1
	1142	63.51	0.00	0.461	-0.112	0.243	0.176	1189.4



	Tr.	<u></u>	_	1~	T	100	<u></u>	_	<u></u>		<u> </u>	100	<u></u>	10	~	<u>~</u>		<u>~</u>	<u> </u>	0	<u> </u>	100	—
HIV PRT GeneChip™ Data	1501.7	1360.9	1112.9	468.3	400.	401.6	199.9	202.	258.7	274.7	297.2	250.6	231.3	214.5	102.3	102.3	49.4	104.3	46.3	50.9	58.2	50.5	53.
Window- Averaged Composite Score	-0.012	-0.156	-0.263	0.353	0.454	-0.544	-0.575	-0.530	-0.491	-0.475	-0.463	0.414	-0.318	-0.286	-0.295	0.330	0.367	0.379	0.370	098'0		Part Control	
Composite Score	-0.192	-0.389	-0.317	-0.572	-0.375	-0.237	-0.387	-0.899	-0.999	-0.552	-0.261	-0.104	-0.119	-0.310	-0.556	-0.324	-0.324	-0.324	-0.353	-0.378	-0.392	-0.494	-0.251
AGMFOLD Score	-0.982	-1.156	-1.156	-1.156	-1.156	-1.417	-1.156	-1.156	-1.156	-1.156	-0.982	-0.373	0.410	0.410	0.410	0.410	0.410	0.410	0.757	1.018	0.670	0.670	0.670
T _m Score	0.291	0.081	0.198	-0.215	0.104	0.485	0.084	-0.741	-0.903	-0.183	0.181	0.061	-0.443	-0.751	-1.147	-0.774	-0.774	-0.774	-1.034	-1.235	-1.043	-1.207	-0.815
∆GMFOLD (kcal/mole @ 35 °C)	-1.00	-1.20	-1.20	-1.20	-1.20	-1.50	-1.20	-1.20	-1.20	-1.20	-1.00	-0.30	09.0	09.0	09.0	09.0	09.0	09.0	1.00	1.30	06.0	06.0	06.0
RNA/DNA Tm (°C)	62.35	60.92	61.71	58.90	61.08	63.68	60.94	55.32	54.21	59.12	61.60	60.78	52.35	55.25	52.55	55.09	55.09	55.09	53.32	51.95	53.26	52.14	54.81
SEQ ID NO:	1143	1144	1145	1146	1147	1148	1149	1150	1151	1152	1153	1154	1155	1156	1157	1158	1159	1160	1161	1162	1163	1164	1165
DNA Probe Sequence	999 TCGTTGCCAAAGAGTGATCT	1000 CGTTGCCAAAGAGTGATCTG	1001 GTTGCCAAAGAGTGATCTGA	1002 TTGCCAAAGAGTGATCTGAG	1003 TGCCAAAGAGTGATCTGAGG	1004 GCCAAAGAGTGATCTGAGGG	1005 CCAAAGAGTGATCTGAGGGA	1006 CAAAGAGTGATCTGAGGGAA	1007 AAAGAGTGATCTGAGGGAAG	1008 AAGAGTGATCTGAGGGAAGT	1009 AGAGTGATCTGAGGGAAGTT	1010 GAGTGATCTGAGGGAAGTTA	1011 AGTGATCTGAGGGAAGTTAA	1012 GTGATCTGAGGGAAGTTAAA	1013 TGATCTGAGGGAAGTTAAAG	1014 GATCTGAGGGAAGTTAAAGG	1015 ATCTGAGGGAAGTTAAAGGA	1016 TCTGAGGGAAGTTAAAGGAT	1017 CTGAGGAAGTTAAAGGATA	1018 TGAGGGAAGTTAAAGGATAC	1019 GAGGGAAGTTAAAGGATACA	1020 AGGGAAGTTAAAGGATACAG	1021 GGGAAGTTAAAGGATACAGT
p5 Probe Position	666	1000	1001	1002	1003	1004	1005	1006	1007	1008	1009	1010	1011	1012	1013	1014	1015	1016	1017	1018	1019	1020	1021



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Example 3

Synopsis: The method of the present invention is particularly useful as a guide to the iterative refinement of probes. One of the specific predictions made for rabbit β-globin in Example 1 is used to provide an example of such a refinement.

Materials and Methods: The contig spanning positions 5-11 of a portion of the rabbit β -globin gene (Example 1, Table 3) was analyzed, using the experimentally measured data to simulate the results of successive experimental measurements. The iterative refinement was performed using a rule-based algorithm, outlined below. This algorithm is used by way of example only; other algorithms for

efficiently finding local maxima are well known to the art and could be employed to

perform this task.

Given experimental data for probes from the 1st quartile, median and 3rd quartile of a contig, as well as a user-set signal threshold for further consideration of a probe,

1) If all 3 measurements are below the user-specified signal threshold, discard the prediction.

 If at least one of the measurements is above the user-specified threshold, determine which point yields the maximum signal.

- a) If the maximum point is the 1st quartile probe, then make three new measurements for probes with the same spacing as that used in the preceding iteration, but displaced so that the third probe is identical to the original 1st quartile probe. In other words, repeat the search with the same pattern and spacing, but displace the pattern in the direction of increasing signal found in the first experiment.
- b) If the maximum point is the 3rd quartile probe, then make three new measurements for probes with the same spacing as that used in the preceding iteration, but displaced so that the first probe is identical to the original 3rd quartile probe. In other words, repeat the search with the same

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- pattern and spacing, but displace the pattern in the direction of increasing signal found in the first experiment.
- c) If the maximum point is the median probe, then repeat the experiment, keeping the median point the same, but shrinking the spacing between probes by a factor of 2.
- 3) Continue iteration until a maximum is found, or the user judges the signal level observed to be acceptable. Use the experimental value measured for the probe duplicated in successive iterations to tie together the successive data sets, via a simple normalization procedure, described below. Where appropriate, consider all of the data (i.e. all of the iterations) when deciding how to proceed, or whether the peak hybridization intensity has been found.

Results: Iterative refinement of the contig spanning positions 5-11 in Table 3 proceeds as follows:

Iteration 1: Probes are synthesized at positions 6, 8 and 10, yielding the experimental hybridization intensities 180, 220 and 310, respectively.

Iteration 2: Following rule 2b), probes are synthesized at positions 10, 12 and 14. Note that the redundant measurement at position 10 serves as a bridge between experiments, and allows comparison of the two sets by normalizing the intensities by multiplying the second iteration measurements by the ratio of the intensity observed for the probe at position 10 in the first iteration to the value observed in the second iteration. In the simplest case, the ratio is 1; in any case, the second iteration yields the normalized values 310, 390, 240 for probe positions 10, 12 and 14, respectively.

Iteration 3: By rule 2c), measurements are performed for probes at positions 11, 12 and 13; after normalization, these yield the normalized hybridization intensities 320, 390 and 410, respectively. Combination of these results with the results from iteration 2, probe position 14, yields the conclusion that the best probe for this intensity peak is the probe that starts at sequence position 13.

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The overall result is that iterative improvement converges in three iterations, and requires the synthesis of seven test probes, one of which is the local optimal probe. In addition, the first and second iterations yield probes that exhibit 75% and 95% of the local maximum hybridization intensities, respectively. In many applications, either of these probes would be considered acceptable.

The above examples 1 and 2 demonstrate that two different implementations of the method of the present invention are capable of efficiently predicting regions of high hybridization efficiency in a variety of polynucleotide targets. Many of the predictions yield acceptable probe sequences on the first design iteration, and all would yield optimized probe sets after 2-4 rounds of iterative refinement, as demonstrated in Example 3. The performance demonstrated in these examples greatly exceeds the performance of current methods. Finally, the examples demonstrate that the predictions can be performed by a software application that has been implemented and installed on a Pentium®-based computer workstation.

All publications and patent applications cited in this specification are herein incorporated by reference as if each individual publication or patent application were specifically and individually indicated to be incorporated by reference.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be readily apparent to those of ordinary skill in the art in light of the teachings of this invention that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims.

```
Private Declare Function GetHomology Lib "homologydll.dll" (ByRef Homologys) As
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      Private Declare Function SkipSequence Lib "homologydll.dll" (ByVal SeqLength&)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       (Byval DB$, Byval NumProbest, Byval We, Byval MaxProbeLengtht) As Long
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    Private Declare Function TerminatePH Lib "homologydll.dll" () As Long
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          Private Declare Function CalcOneHomology Lib "homologydll.dll" (ByVal

    This structure holds read from one sequence file/DB/website.
    It it used when reading Genbank files (in which case all fields
    are filled in), or FASTA files (in which case only the Header and

                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               Private Declare Function AddProbe Lib "homologydll.dll"
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      Private Declare Function InitPH Lib "homologydll.dll"
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               (ByVal Probes, ByVal ProbeLength&) As Long
                             Public ClampPars As New cClampPars
Public ClampFilterPars As New cClampFilterPars
                                                                                                                  Public HomologyPars As New CHOmologyPars
Public HomoFilterPars As New CHOmoFilterPars
Public ArrayPars As New CArrayPars
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         'Initialize thermodynamic parameters.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  Public Const MaxBLASTHeaders& = 100

    Sequence portions are filled in).

                                                                                           Public BlastPars As New CBLASTPars
Public TrimPars As New cTrimPars
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   Copyright Hewlett-Packard Company, 1998
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           Attribute VB_Name = "Blast"
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 Accession As String
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               Sequence As String
                                                                                                                                                                                                                                                           SegRecord Structure
                                                                                                                                                                                                                                                                                                                                                                                                                  Public Type SeqRecord
                                                                                                                                                                                                                                                                                                                                                                                                                                                   Header As String
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              SeqLength&) As Long
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                Locus As String
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  Length As Long
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           Option Explicit
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      InitThermoPars
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 *Start 1t up!
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     frmMain.Show
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           Sub Main()
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             End Type
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             End Sub
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   Attorney Docket No. 10971464-1
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                workspace DB template workspace DBs directory
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              workspace DB extension
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            Genbank directory
Genbank file extension
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 'BLAST databases
                                                                                                                                                                                                                                                                                                                                                                                                                                                   application key
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               'Flags to prevent sequence and probeset selectors firing. 'Currently broken -- RowColChange events are ignored. Public AllowdbgClick As Boolean
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             Public PosfilterPars As New cPosfilterPars
Public LengthFilterPars As New cLengthFilterPars
                                                                                                                                                                                                                                                                                                                                                                                                                                               Public Const SKEY PPPPP$ = "PamelaProbeDesigner"
Public Const VALUE_DB_TEMPLATE$ = "DBTemplate"
Public Const VALUE_DB_DIR$ = "DBD1"
Public Const VALUE_DB_PILE_EXT$ = "DBF1leExt"
Public Const VALUE_GB_DIR$ = "GBD1"
Public Const VALUE_GB_FILE_EXT$ = "GBD1"
Public Const VALUE_GB_FILE_EXT$ = "GBF1leExt"
Public Const VALUE_BB_FILE_EXT$ = "GBF1leExt"
                                                                                                                                                             Public Const DBRev$ = "PP MDB Template 1.04"
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              Public RunPars As New CRunPars
Public RunFilterPars As New CRunFilterPars
Public Progress As New CProgress
Public EntrezPars As New CENTrezPars
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    Public TWFilterPars As New cTMFilterPars
Public dGHPars As New cDGHPars
Public dGHFilterPars As New cdGHFilterPars
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        Public dGDPars As New cdGDPars
Public dGDFilterPars As New cdGDFilterPars
Public TMPars As New CTMPars
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                Public dGMFilterPars As New cdGMFilterPars
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           Public GCFilterPars As New cGCFilterPars
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         Public CreatePSPars As New cCreatePSPars
                                                                                                  'Program and DB Rev
Public Const ProgRev$ = "PP Rev 1.00"
                                                                                                                                                                                                                                                           Public PSColumns As New Collection
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         Copyright Hewlett-Packard Company, 1998
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            ppErrNoProbesCreated = 3003
ppErrCancelled = 3004
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   Public dGMPars As New cdGMPars
                                                                                                                                                                                                                                                                                                                                                    Public StatArray As New XArray
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    ppErrBlastVersion = 3006
ppErrFASTAFormat = 3007
ppErrSeqLoadDB = 3008
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 ppErrGBFileLength = 3000
ppErrGBFileFormat = 3001
ppErrBadRecordset = 3002
   Attribute VB_Name = "General"
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  'Algorithm parameter sets
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          ppErrDBFormat - 3005
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       ppErroll = 3009
                                                                                                                                                                                                                           Columns collection
                                                                                                                                                                                                                                                                                                                   Statistics XArray
                                   Option Explicit
                                                                                                                                                                                                                                                                                                                                                                                                                     Registry keys
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             Enum ppErrors
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               Error codes
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     directory
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'list of accession numbers not to check homology against te
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   'Read the number of entries expected. The progressbar for this application 'is posted with a maximum of 100, since we don't know the number of sequences
Public Sub CalcHomology(Seq$(), HP As CHomologyPars, Homology&(), RSBlast As Recordset, PSName$)
                                                                                                                                                       Calculate the homology of an array of probes to a database of sequences.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  'is homology to be done for this sequence
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                'Initialize the homologizer.
If (IniterHer.Park & N. & HomologyBaseName & ".seqs", NumSeqs, HP.Seed,
MaxProbelen) <> 0) Then
Err.Raise pperrdll, "Error in InitPH DLL Call."
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               Open HP.Path & "\" & HomologyBaseName & ".headers" For Input As #DBFile
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   Determine the number of probes we are calculating homology for
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       If Len(Seq(P)) > MaxProbeLen Then MaxProbeLen = Len(Seq(P))
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        'number of sequences we are working with
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              "Check for the existence of the homologizer files. HomologyBasseName = Left (HR.DB, Left (HR.DB, Left (HR.DB, Left (HR.DB, Left (HR.DB, Left (HR.DB))) = 4] foo = FileLen(HP.Path & "\" & HomologyBaseName & ".seqs") foo = FileLen(HP.Path & "\" & HomologyBaseName & ".seqs")
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 Err.Raise pperrdll, , "Error in AddProbe Dil Call."
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       Dim DBF11e.

Dim SR As SeqRecord one header from the header file
Dim LineText; 'one line from the header file
Dim NumHeaderse; 'one line from the header file
Dim NumHeaderse; 'number of headers in the header file
Dim DoHomology As Boolean '1s homology to be done fo
Dim No. NumNoCheck;
Dim NoChecks() 'list of accession numbers not to check
                                                                                                                                                                                                                                                                                                Homology: The homologies.
RSBLast: The table of BLAST-located homologies.
PSName: The name of the probeset we are processing.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              'Load the probes into the homologizer index.

For P = 0 To NumSeqs - 1

If (AddProbe(Seq(P), Len(Seq(P))) <> 0) Then
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          ·longest probe
                                                                                                                                                                                                                        Seq: The sequences.
HP: The homology parameters.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        · 1ndex
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     NumSeqs - UBound (Seq) + 1
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     For P = 0 To NumSeqs - 1
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         'Find the longest probe.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          Open the specified DB
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               Dim HomologyBaseName
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                DBF1le = FreeFile
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      Dim MaxProbeLen∉
Dim P£, S‡
                                                                                                                                                                                                                                                                                                                                                                                                                                                On Error GoTo E
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   MaxProbeLen = 0
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        Dim NumSeqs6
                                                                                                                                                                                             Arguments
                                                                                                                     Funct 1on
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             Next P
```

```
'Create an array of all accessions that should not be searched against.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     If (CalconeHomology(SR.Length) <> 0) Then __
Err.Raise pperrdll, , "Error in CalconeHomology DLL Call."
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         If (SkipSequence(SR.Length) <> 0) Then _____Err.Raise pperrdll, , "Error in SkipSequence DLL Call."
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    If (Fix(100 * S / NumHeaders) - Progress.StopAt > 0) Then Progress.CheckProgress Fix(100 * S / NumHeaders) 'Read the next DB sequence.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       If SR.Accession = Nocheck(N) Then DoHomology = False Next N
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            If (GetHomology(Homology(0)) <> 0) Then Err.Raise pperrdll, ' "Error in GetHomology DLL Call."

If (TerminatePH() <> 0) Then
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                Err.Raise pperrdll, , "Error in TerminatePH DLL Call."
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             'Check the header against the bad list.
DoHomology = True
For N = 0 To NumWoCheck - 1
                                                                                                                                                                                                                                                                                                                                                                                                                 If Not RSBlast("Use") Then
NoCheck(N) = RSBlast("Accession")
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   E: Debug.Print *Error in CalcHomology*
Err.Raise Err.Number, , Err.Description
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        'Homologize all the sequences in the DB
                                                                                                                                                                                                                                                                      NumNoCheck = RSBlast.RecordCount
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          'Retrieve the answers, and clean up.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       SR = ReadFASTAHeader (DBF11e)
                                                                                                                                                                                                                                                                                                                             ReD1m NoCheck (0 To NumNoCheck)
'in advance of opening the DB.
Line Input #DBFile, LineText
                                                       NumHeaders = CLng(LineText)
                                                                                                                                              NumNocheck = 0
If IsGoodRS(RSBlast) Then
                                                                                                                                                                                                                                                                                                                                                                                     Do While Not RSBlast . EOF
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                Do While Not EOF (DBFile)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             If DoHomology Then
                                                                                                                                                                                                                                          RSBlast.MoveFlrst
                                                                                                                                                                                                        RSBlast.MoveLast
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      RSBlast.MoveNext
                                                                                                                                                                                                                                                                                                                                                                                                                                                                               Z + Z = Z
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 NumNocheck . N
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              Do 1t.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                End If
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         9007
```

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Attorney Docket No. 10971464-1
                                                                                                                                                                            BOFILENAme = PSName & ".out"

cmd = "cmd /c cd " & BP.Path

cmd = cmd & " & blastall -p blastn -1 " & BIFILEName & " -d " & BLASTBaseName &

" -o " & BOFILENAME
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             0
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             Loop Until InStr(LineText, "Sequences producing significant alignments:") <>
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 Line Input #BOFile, LineText If Left (LineText, 12) <> "BLASTN 2.0.2" Then Err.Raise ppErrBlastVersion
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      Open BP.Path 6 "\" & BOFileName For Input As #BOFile
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        LineText = Right (LineText, Len(LineText) - 68)
MatchScores (H) = CDB/Lieft(LineText, 4))
LineText = Right(LineText, Len(LineText) - 4)
If isNumeric(LineText) Then
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             'Read match information, until a blank line
                                                                                                                                                   Put together the command string to execute
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                'Read as many headers as we found matches.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   MatchExpects(H) = CDbl(LineText)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     "We will return the number of matches
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           9
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   Line Input #BOFile, LineText
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 'Strip lines until We see ">".
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            On Err GoTo ErrNoSignificantMatch
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      Line Input #BOFile, LineText
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           © Copyright Hewlett-Packard Company, 1998
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 Line Input #BOFile, LineText
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     MatchExpects(H) = 0#
End If
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      Loop Until Len(LineText) = 0
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  Line Input #BOFile, LineText
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      Line Input #BOFile, LineText
                                                                                                                                                                                                                                                                                                                                                                  ProcID = Shell (cmd, vbHide)
                                                                                                                                                                                                                                                                                                                                                                                               foo = WaltOnProgram(ProcID)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     *Check for correct version
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            *Move down to MatchExpects
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            For H = 0 To BLASTPS - 1
Print #BIFile, Sequence
                                                                                                                                                                                                                                                                                                                                                                                                                                                              'Open the output file.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         'Strip one more line
                                                                                                                                                                                                                                                                           -o * & BOFileName
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             BOFile = FreeFile
                                                           'Close input file.
                                                                                                                                                                                                                                                                                                                                      'Run it and wait.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             On Error GoTo E
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    BLASTPS = H - 1
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          H = H + 1
                                                                                           Close BIFile
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      0
1
H
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              Attorney Docket No. 10971464-1
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             'Check for the existence of all the BLAST files. If files are absent, an error
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      'One line of the BLAST output file.
Index the matches.
Location of the GenBank accession within the
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          This is a very fragile function, since it depends on the format of the BLAST output file (checked only for version number), and relies on every header containing an accession number (though one will be manufactured
                                                                                                                                                      Run BLAST on the sequence of a probeset, saving headers and scores of
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         *BLAST input and output filles.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 Process ID of running BLAST program.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  the root name for the BLAST files. *BLAST command string.
      Public Function BLASTPS& (PSName$, ByVal Sequence$, BP As cBLASTPars,
                                                                                                                                                                                                                                                                                                                                                                                                                                 MatchExpects: The expected number of matches with such scores.
                                                                                                                                                                                                                                              PSName: The probeset name, used to name temporary files
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  BLASTBaseName = Left (BP.DB, Len (BP.DB) - 4)
foo = Fileten (BP.Path & "blastall.exe")
foo = Fileten (BP.Path & "" & BLASTBaseName t ".nin")
foo = Fileten (BP.Path & "" & BLASTBaseName t ".nhr")
foo = Fileten (BP.Path & "\" & BLASTBaseName t ".nhr")
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        Open BP. Path & "\" & BIF1leName For Output As BIF1le
                                        Do While Len(Sequence) > 60
Print #BIFile, Mid$(Sequence, 1, 60)
Sequence = Right(Sequence, Len(Sequence) - 60)
                                                                                                                                                                                                                                                                              Sequence: The base sequence of the probeset
                                                                                                                                                                                                                                                                                                                                MatchAccession: Matching accession numbers.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   Bit bucket
                                                                                                                                                                                                                                                                                                                                                                  MatchHeader: The full matching header. MatchScores: The match scores.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   Print #BIFile, "> PSName = " & PSName
                                                                                                                                                                                                                                                                                                          BP: The BLAST search parameters.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 @ Copyright Hewlett-Packard Company, 1998
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            Dim BifileName$, BOFileName$
Dim Bifilet, BOFilet
Dim BLASTBaseName$
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     BIFileName = PSName & ".fsa"
BOFileName = PSName & ".out"
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                Open the BLAST input file
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  "Write out the sequence.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          'Write out the header.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           if none is found).
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        *BLAST file names.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            BIF11e - FreeFile
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    On Error GoTo E
                                                                                                                                                                                            homologues.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             Dim Procid
Dim foo
Dim LineText$
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          match header.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        Dim GBWhere&
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  occur.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       D1m cmd$
```

```
TMValid = TMValid And TMPars.Validate(RSPS) And TMFilterPars.Validate(RSPS)
dGHValid = dGHValid And dGHPars.Validate(RSPS) And
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  CreatePSValid = CreatePSValid And CreatePSPars.Validate(RSPS)
LengthFilterValid = LengthFilterValid And LengthFilterPars.Validate(RSPS)
PosFilterValid = PosFilterValid And PosFilterPars.Validate(RSPS)
                                                                                                                                                                                       3. Filter columns are always valid, since they are initialized with True.
1. A column is valid if the values of the parameters used to calculate it are equal to the values of those parameters in the current instance of the appropriate parameter class.

2. This calculations is settings in the global state variable PSColumns. It should therefore only be called on the Probesets and Probes used to build dbgProbes, i.e. datsalps.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            GCF11terValid = GCF11terValid And GCF11terPars.Validate(RSPS) dGDValid = dGDValid And dGDPars.Validate(RSPS) And
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  If Not IsGoodRS(RSPS) Then
For Each Col In PSColumns
    If Col.CanBeInValid Then Col.IsValid = False
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         'If the recordsets are empty, then nothing is valid
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               'On startup, no columns exist, so bail.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           If PSColumns.Count = 0 Then Exit Sub
                                                                                                                                                                                                                                                                                                       Dim LengthFilterValid As Boolean
Dim PosFilterValid As Boolean
Dim GCFilterValid As Boolean
Dim GCFilterValid As Boolean
Dim GGDValid As Boolean
Dim TMValid As Boolean
Dim GGHValid As Boolean
Dim GGMValid As Boolean
Dim ClampValid As Boolean
Dim HomolayValid As Boolean
Dim HomolayValid As Boolean
Dim Col As Ccolumn
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          C Copyright Hewlett-Packard Company, 1998
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       dGDF11terPars.Val1date(RSPS)
                                                                                                                                                                                                                                                                              Dim CreatePSValid As Boolean
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 dGHF11terPars.Val1date (RSPS)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          LengthFilterValid - True
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   'Set everything valid.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   PosFilterValid = True
GCFilterValid = True
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         Do While Not RSPS.ECF
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  True
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                HomologyValid = True
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              'Try to invalidate.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    ClampValid = True
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         dGMValid = True
RunValid = True
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               CreatePSValid =
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  dGDValld = True
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                dGHValid = True
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 TMValld = True
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          RSPS.MoveF1rst
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            Exit Sub
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            Next Col
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       End If
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          Attorney Docket No. 10971464-1
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       MatchAccession(H) = MatchAccession(H) & Mid(MatchHeader(H), GBWhere,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        RSPS: The parameter recordset for the ProbeSets being checked
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    'No match was found, so we've skipped over the match information. BLASTPS = 0
                                                                                                                                                           "Keep pushing on left-justified lines until we see "Length".
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              Loop Until (Mid(MatchHeader(H), GBWhere, 1) = " ")
                                                                                                                                                                                                                                                  Public sub ColumnsValidate (RSPS As Recordset)
                                                                                                                                                                                                                                                                                                                                                                                                                                   GBWhere = InStr(MatchHeader(H), "/gb=")
If (GBWhere = 0) Then
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      Err.Raise Err.Number, , Err.Description
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               Attribute VB_Name = "ColumnValidation"
         Loop Until Left(LineText, 1) = ">"
                                                                                                                                                                                                                         Line Input #BOFile, LineText
                                                                 *Push this line onto the header. MatchHeader(H) = LineText
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        MatchAccession(H) = "200000"
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             Check the validity of columns.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  GBWhere - GBWhere + 1
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       E: Debug.Print "Error in BLASTPS"
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          @ Copyright Hewlett-Packard Company, 1998
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  Kill BP.Path & "\" & BIFileName Kill BP.Path & "\" & BOFileName
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         Kill BP.Path 6 "\" & BIFileName Kill BP.Path 8 "\" & BOFileName
                                                                                                                                                                                                                                                                                                                                                                                                    'Find the accession number.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            GBWhere - GBWhere + 4
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    Remove the BLAST files.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               ErrNoSignificantMatch:
                                                                                                                                                                                                                                                                                                                                               Loop Until False
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              Option Explicit
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    Close #BOFile
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       Exit Function
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         Close #BOFile
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                Exit Function
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     End Function
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           BLASTPS = 0
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               Arguments
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 Funct 1on
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         Next H
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   7
```

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A. For each probeset, the appropriate existence parameter is set. This indicates that some calculation has been performed to fill in the column, at sometime in the past. However, it is possible that
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  frmMain.dbgProbes.Columns(Col.Name).HeadForeColor = vbBlack
                                                                                                                                                                                                                        ClampFilterPars.Validate(RSPS)
HomologyValid - HomologyValid And HomologyPars.Validate(RSPS) And HomoFilterPars.Validate(RSPS)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              PSCOlumns ("Sequence") . IsValid = CreatePSValid
PSCOlumns ("Sequence") . IsValid = CreatePSValid And LengthFilterValid
PSCOlumns ("Length Filter") . IsValid = PSCOlumns ("Length") . IsValid
PSCOlumns ("Position") . IsValid = CreatePSValid And PosFilterValid
PSCOlumns ("Pos Filter") . IsValid = CreatePSValid And PosFilterValid
PSCOlumns ("Pos Filter") . IsValid = PSCOlumns ("Fosition") . IsValid
PSCOlumns ("GC Filter") . IsValid = PSCOlumns ("GC") . IsValid
PSCOlumns ("GD Filter") . IsValid = PSCOlumns ("Duplex dG") . IsValid
PSCOlumns ("ADUPLEX "DIVALID = PSCOlumns ("TM") . IsValid
PSCOlumns ("TM Filter") . IsValid = PSCOlumns ("TM") . IsValid
PSCOlumns ("GGH") . IsValid = PSCOlumns ("TM") . IsValid
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          frmMain.dbgProbes.Columns(Col.Name).HeadForeColor = vbRed
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 PSColumns("Homology Filter"). IsValid = PSColumns("Homology"). IsValid
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  PSCOlumns ("dGM Filter") .IsValid = PSCOlumns ("dGM") .IsValid PSCOlumns ("Run Length") .IsValid = RunValid PSCOlumns ("Run Filter") .IsValid = PSCOlumns ("Run Length") .IsValid PSCOlumns ("Clamp") .IsValid = ClampValid
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           Public Sub ColumnsExist (RSPS As Recordset, RSProbes As Recordset)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         PSColumns ("Clamp Filter"). IsValid = PSColumns ("Clamp"). IsValid
                                                                                                                                                                              ClampVal1d = ClampVal1d And ClampPars.Val1date(RSPS) And
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   PSCOlumns ("dGH Filter"). IsValid = PSCOlumns ("dGH"). IsValid PSCOlumns ("dGM"). IsValid = dGMValid
                                         dGMFilterPars.Validate(RSPS)
RunValid = RunValid And RunPars.Validate(RSPS) And
dGMValld = dGMValld And dGMPars.Valldate(RSPS) And
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             'Set column heading backcolor according to validity.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       SColumns ("Homology") . IsValid = HomologyValid
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        RSPS: The probeset(s) parameter recordset
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 Check the existence of columns.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 PSProbes: The probeset probes.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  For Each Col In PSColumns
If Col.CanBeInValld Then
                                                                                                                                     RunFilterPars.Validate(RSPS)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         If Col. IsValld Then
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         1. A column exists if
                                                                                                                                                                                                                                                                                                                                                                      RSPS.MoveNext
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       Update columns.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     Else
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null values is necessary and sufficient; however, it is potentially time-consuming to check, so condition A is checked first, and condition B is checked only if A passes.
the selection criteria have changed since the calculation, bringing into scope probes that have not had the calculation performed. Thut the existence parameter is necessary, but not sufficient.

B. For each probe, the appropriate value is not NULL. A lack of

    This calculation affects settings in the global state variable
PSColumns. It should therefore only be called on the Probests and
Probes used to build dbgProbes, 1.e. datSelPS and datSelPQuery.
    Filter columns always exist, since they are initialized with True.

                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            'If the recordsets are empty, then nothing exists, nothing is valid.

If Not (ISGOOGRS(RSFS) And ISGOOGRS(RSProbes)) Then

For Each Col In PSColumns

If Col.CanUnExist Then Col.Exists = False
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        Do While Not RSPS.EOF
Exists - Exists And RSPS("CreatePS-Exists")
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                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   Exists - Exists And RSPS("TM-Exists")
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           On startup, no columns exist, so bail.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  PSColumns ("Accession") . Exists - Exists
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    PSColumns ("PSName"). Exists = Exists
PSColumns ("Sequence"). Exists = Exists
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        PSColumns ("Position") . Exists = Exists
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                If PSColumns.Count = 0 Then Exit Sub
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                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     PSColumns ("GC") . Exists = Exists
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                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   Probe creation calculations.
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                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  Dim Exists As Boolean
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Attorney Docket No. 10971464-1

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Attorney Docket No. 10971464-1
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                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      2. The calling routine is responsible for adequately dimensioning Probes,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            StartPos = 1
ProbeNum = LBound(Probes)
Do While StartPos <= Len(Seq) - CPSP.Length + 1
If ProbeNum - Progress.StopAt = 0 Then Progress.CheckProgress StartPos Probes(ProbeNum) = Mid(Seq, StartPos, CPSP.Length)
Positions(ProbeNum) = StartPos
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  Public Function CreatePSEqualTM& (Seq$, CPSP As cCreatePSPars, Probes$(), _
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        CPSP: An instance of the parameter class for this algorithm. Probes: The array in which the probes will be returned. Positions: The array in which the starting positions will be returned. GC: The array in which GC content will be returned.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            CPSF: An instance of the parameter class for this algorithm. Probes: The array in which the probes will be returned. Posttions: The array in which the starting positions will be returned. GC: The array in which GC content will be returned.
                                                                                                                                                                                                                                                                                                                                                                                                                                                   Public Function CreatePSEqualL&(Seq$, CPSP As cCreatePSPars, Probes$(), __
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             Seq: The sequence from which the probes are created.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          Create a set of probes using the EqualLength method
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         Seq: The sequence from which the probes are created
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         Create a set of probes using the EqualIM method.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             GC(ProbeNum) = DNA_GCContent (Probes (ProbeNum))
StartPos = StartPos + CPSP.Spacing
ProbeNum = ProbeNum + 1
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         the starting position of a probethe current probe
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                                         PSColumns ("Homology") . Exists = Exists
                                                                                                                                                                                                                                                                                                           Attribute VB_Name = "CreateProbeSet"
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         The number of probes created.
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                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  Positions&(), GC#())
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If Not RSProbes.NoMatch Then Exists = False
                                                                                                                                                                                            RSProbes.FindFirst "dGH = NULL"
If Not RSProbes.NoMatch Then Exists = False
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      If Not RSProbes. NoMatch Then Exists - False
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Exists = Exists And RSPS("Homology-Exists")
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 If Not RSProbes.NoMatch Then Exists - False
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            If Not RSProbe.NoMatch Then Exists - False
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Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists = Exists =
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                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           Do While Not RSPS.EOF
Exists = Exists And RSPS("dGM-Exists")
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      Do While Not RSPS.EOF
Exists = Exists And RSPS("Run-Exists")
Exists = Exists And RSPS("dGH-Exists")
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  RSProbes.FindFirst "[Clamp] = NULL"
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   RSProbes.FindFirst "dGM = NULL"
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                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     PSColumns ("dGM") . Exists = Exists
                                                                                                                                                                                                                                                                                                                                               PSColumns ("dGH") . Exists = Exists
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        C Copyright Hewlett-Packard Company, 1998
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   Run Length Calculations.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              'Homology Calculations
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   If Exists = True Then
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   If Exists - True Then
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              Exists = True Then
                                                                                                                                                  If Exists - True Then
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        Clamp Calculations.
                                                                                                                                                                                                                                                                                                                                                                                                                                                        'dGM Calculations.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          RSPS.MoveNext
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         RSPS.MoveNext
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               RSPS.MoveNext
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               RSPS.MoveNext
                                                    RSPS.MoveNext
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  RSPS.MoveFirst
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            RSPS.MoveFlrst
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 RSPS.MoveFirst
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            RSPS.MoveF1rst
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  Exists = True
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       Exists = True
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             Exists - True
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  Exists - True
```

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Attorney Docket No. 10971464-1
If (Mid(Seq, 1, 1) = "g" Or Mid(Seq, 1, 1) = "c") Then DNA_GCContent = DNA_GCContent + 1
                                                                                                                                                                                                                                                                                                If Bases < Len(Seq) Then
If WhichEnd = "5'" Then DNA_SeqTrim = Right(Seq, Len(Seq) - Bases)
If WhichEnd = "3'" Then DNA_SeqTrim = Left(Seq, Len(Seq) - Bases)</pre>
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         MsgBox "Unknown base found while calculating DNA_RevComp."
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         If WhichEnd = "5" Then DNA SeqKeep = Left (Seq, Bases) If WhichEnd = "3" Then DNA SeqKeep = Right (Seq, Bases)
                                                                                                                                                                                                                                                                                                                                                                                                                                                          Public Function DNA_SeqKeep$(Seq$, Bases&, WhichEnd$)
                                                                                                                                                                    Public Function DNA_SeqTrim$ (Seq$, Bases&, WhichEnd$)
                                                                                                                                                                                                                                                   Trim off one end or the other of the sequence.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 28-Jul-97: From PkWs routine of the same name.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    Given 5'-3' strand, returns 5'-3' complement.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    Remove all but the specified number of bases,
                                                                                       DNA_GCContent = DNA_GCContent / Len(Seq) * 100
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              Case "a"

DNA RevComp = DNA_RevComp 6 "t"

Case "c"
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    DNA_RevComp = DNA_RevComp & "a"
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          Public Function DNA_RevComp$(ByVal Seq$)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  DNA_RevComp = DNA_RevComp & "g"
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  DNA_RevComp - DNA_RevComp & "C"
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    DNA_RevComp = DNA_RevComp & "a"
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      DNA_RevComp = DNA_RevComp & "n"
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 7
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 © Copyright Hewlett-Packard Company, 1998
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                Select Case Mid$ (Seq, 1, 1)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              Seq = LCase(Seq)
For 1 = Len(Seq) To 1 Step -1
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   DNA RevComp = Seq
Exit Function
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      If Bases <= Len(Seq) Then
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        End Select
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   Case Else
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            Case "t"
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             End Function
                                                                                                                                                                                                                                                                                                                                                                                                       End Function
                                                                                                                       End Function
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    case '
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            Function
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               'Function
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    Case
                                                                                                                                                                                                                           Function
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               Revision'
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               Case
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            DIM 16
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    Attorney Docket No. 10971464-1
                                                                                                                                                                                                                                                                                                                           If Probenum - Progress.StopAt = 0 Then Progress.CheckProgress StartPostreate the probes, extending until melting point is above target.
                                                                                               the starting position of a probe
the current brobe
current length of the probe being created
current TM of the probe being created
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          TM - DNA CalcTM(Probes(ProbeNum), CPSP.Conc)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 TM = DR_CalcTM(Probes(ProbeNum), CPSP.Conc)
End Select
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          Probes (ProbeNum) = Mid (Seq, StartPos, Length)
Select Case CPSP.Duplex
Case "DNA/DNA"
                                                                                                                                                                                                                                                                                                                                                                                                                                                                         TM = DNA_CalcTM(Probes(ProbeNum), CPSF.Conc)
Case "DNA/RNA"
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    TM = DR_CalcTM(Probes(ProbeNum), CPSP.Conc)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      GC(ProbeNum) = DNA_GCContent(Probes(ProbeNum))
ProbeNum = ProbeNum + 1
                                                                                                                                                                                                                                                                                                                                                                                                          Probes(ProbeNum) = Mid(Seq, StartPos, Length)
Select Case CPSP.Duplex
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         Length = Length + 1

Do While TW < CREP.Target

If StartPos + Length > Len (Seq) Then

ProbeNum = ProbeNum = 1
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            Public Function DNA_GCContent (ByVal Seq$)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      Compute the GC content of a sequence.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            Attribute VB_Name = "DNA_Manipulations"
Option Explicit
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           StartPos = StartPos + CPSP.Spacing
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       Ξ
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           Positions (Probenum) = StartPos
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       Copyright Hewlett-Packard Company, 1998
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        CreatePSEqualTM = ProbeNum - 1
End Function
                                                                                                                                                                                                                           StartPos = 1
ProbeNum = LBound(Probes)
Do While StartPos <= Len(Seq)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 Length = Length + 1
                       Positions, and GC.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          DNA_GCContent = 0
For 1 = 1 To Len(Seq)
                                                                                                                                                                                                                                                                                                           Check progress
                                                                                                                                                                                                                                                                                                                                                                                                                                                               Case "DNA/DNA"
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     Seq - LCase (Seq)
                                                                                                                                                                                                                                                                                                                                                                                       Length = 1
                                                                                                    Dim StartPose
                                                                                                                            Dim ProbeNume
Dim Lengthe
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   Funct 1on
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             D1m 16
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ogyensy4 deleni

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Attorney Docket No. 10971464-1
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        Given 5'-3' strand, returns 3'-5' sequence. This might be useless!
                                               Given 5'-3' strand, returns complement. This might be useless!
                                                                                                                                                                                                                                                                                                                                                                                                        MsgBox "Unknown base found while calculating DNA_Comp."
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             MsgBox "Unknown base found while calculating DNA_Rev."
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    15
Public Function DNA_Comp$(ByVal Seq$)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      Public Function DNA_Rev$ (ByVal Seq$)
                                                                                                                                                                                                                                     DNA_Comp = DNA_Comp & "c"
                                                                                                                                                                                DNA_Comp = DNA_Comp & "t"
                                                                                                                                                                                                                    DNA_Comp = DNA_Comp & "g"
                                                                                                                                                                                                                                                                                             DNA_Comp = DNA_Comp & "a"
                                                                                                                                                                                                                                                                                                                                 DNA_Comp = DNA_Comp 6 a
                                                                                                                                                                                                                                                                                                                                                                      DNA_COMP = DNA_COMP 4 "n"
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    © Copyright Hewlett-Packard Company, 1998
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            Seq = LCase(Seq)
For 1 = Len(Seq) To 1 Step -1
Select Case Mid$(Seq, 1, 1)
Case "a"
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      DNA_Rev = DNA_Rev & "C"
Case "g"
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                DNA Rev = DNA Rev & "t"
Case "u"
                                                                                                   Seq = Lcase(Seq)
For 1 = 1 To Len(Seq)
Select Case Mid$(Seq, 1, 1)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             DNA Rev = DNA Rev & "g"
Case "t"
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     DNA_Rev = DNA_Rev & "a"
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      DNA_Rev - DNA_Rev & "u"
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          DNA_Rev = DNA_Rev & "n"
                                                                                                                                                                                                                                                                                                                                                                                                                             DNA_Comp = Seq
Exit Function
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  DNA Rev = Seq
Exit Function
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        End Select
                                                                                                                                                                                                                                                                                                                                                                                                                                                                 End Select
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               End Function
                                                                                                                                                                                                                                                                                                                                                      Case "n"
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      End Function
                                                                                                                                                                Case "a"
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           'Funct 1on
                                                                                                                                                                                                                                                                                                                Case
                                                                                                                                                                                                     Case
                                      · Funct 1on
                                                                                           Dim 16
```

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MsgBox "Unknown numeric code found while calculating DNA_Num2str."
End Select
                                                                              'Arguments
'Seq: The original sequence.
' Seq: The original sequence. w/ lower bound 0.
' NumSeq: An integer array representing the sequence, w/ lower bound 0.

    NumSeq must be correctly dimensioned by the calling routine.
    since VB won't let me return an array from a function.

                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         MsgBox "Unknown base found while calculating DNA_Str2Num." End Select
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        NumSeq: An integer array representing the sequence.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              Return the string representation of the DNA string
                                                           Return a numeric array representing the DNA string
Public Sub DNA_Str2Num(ByVal Seq$, ByRef NumSeq*())
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 DNA_Num2str = DNA_Num2str 6 *t* Case *-1*
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            DNA_Num2Str = DNA_Num2Str 6 "a"
Case "1"
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         DNA_Num2str = DNA_Num2str 6 "c"
Case "2"
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               DNA_Num2Str = DNA_Num2Str 6 "g" Case "3"
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        DNA_Num2Str = DNA_Num2Str 6 "n"
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   Public Function DNA_Num2str$(NumSeq*())
                                                                                                                                                                                                                                                    Seq = LCase(Seq)
For 1 = 0 To Len(Seq) = 1
Select Case MId(Seq, 1 + 1, 1)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     For 1 = 0 To UBound (NumSeq)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             Select Case NumSeq(1)
Case *0*
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       NumSeq(1) = -1
                                                                                                                                                                                                                                                                                                                                                                                                       Case "g"
NumSeq(1) = 2
                                                                                                                                                                                                                                                                                                                                                                                                                                                                      NumSeq(1) = 3
                                                                                                                                                                                                                                                                                                                                       NumSeq(1) = 0
                                                                                                                                                                                                                                                                                                                                                                                   NumSeq(1) = 1
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              NumSeq(1) = 3
                                                                                                                                                                                                                                                                                                                                                                                                                                                Case "t"
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      Case "n"
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          Case "u"
                                                                                                                                                                                                                                                                                                                    Case "a"
                                                                                                                                                                                                                                                                                                                                                               Case "c"
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      'Arguments:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               Funct 1on:
                                           Funct 1on
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          Next 1
End Sub
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Attorney Docket No. 10971 464-1
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    Under normal use (e.g. when called by the GUI), this routine uses the
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      'RSSeq: The sequence recordset (positioned to current sequence).
RSPS: The Probestes recordset (used to add a new Probess).
RSProbes: The Probes recordset (used to add new probes).
RSPS: The Parameters recordset (used to add a new parameter record).
                                                                                                                                                                                                                                                                                                                                  Private Sub SeqCalcCreatePS(RSSeq As Recordset, RSProbes As Recordset,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               values in CreatePSPars. 2. Only RSSeq must be a good recordset, since all the others are used only to add records.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  ppErrNoProbesCreated: Raised if no probes are generated.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    'temporary store for created probes 'temporary store for created positions 'temporary store for GC content 'number of probes created
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     current sequence length
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         'index variable for traversing Probes
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 text for progress bar
                                                                                                                                                                                                                           E: Debug.Print "Error in PSCalcBlast"
Err.Raise Err.Number, , Err.Description
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             'new Probeset name
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             'current accession
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             8
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     .current sequence
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             If Not IsGoodRS(RSSeq) Then Exit Sub
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    'Get the sequence, accession, etc. Accession = RSSeq("Accession")
                                                                                                                      'Store parameters in the database.
BlastPars.StoreDB RSPS
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             © Copyright Hewlett-Packard Company, 1998
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 Seq = RSSeq("Sequence")
SeqLength = RSSeq("Length")
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    NumPS = RSSeq("NumPs")
                                                                                                                                                                                                                                                                                                                                                            RSPS As Recordset)
                                                                                                                                                                                                                                                                                                                                                                                                                                            Create a ProbeSet
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        'Check the recordset
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           New probeset name.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  Dim NumPst
Dim PSName$
Dim Probes$()
Dim Positionst()
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      Dim ProgressStr$
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             Errors Handled
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      On Error GoTo E
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  Errors Raised
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         Dim GC#()
Dim NumProbes6
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             Dim Accession$
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             Dim SeqLengths
End With
                                                                               UpdateOnly:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                    *Arguments
                                                                                                                                                                                                                                                                                                                                                                                                                 Funct 1on
                                                                                                                                                                                                           Exit Sub
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               Notes
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              Dim P.
                             Next H
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                Attorney Docket No. 10971464-1
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 Progress.ShowProgress "Calculating BLAST matches for Probeset " & PSName, 0, 1
NumMatches = BLASTPS(PSName, Sequence, BlastPars, MatchAccession, MatchHeaders,
MatchScores, MatchExpects)
                                                                                                                                                                                                                                                                                             RSPS: The parameters recordset (set parameters used in current record).
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             .Fields("Score") = MatchScores(H)
.Fields("Expect") = MatchExpects(H)
If MatchScores(H) > BlastPars.SCutoff Or MatchExpects(H) <
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       'Check for this record already being present.
FindFirst "PSName = '" & PSName 6 "' AND Accession = '" &
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      'scores of matches
'expected value of matches
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      loop index
number of matches found
                                                                                                                                                              Private Sub PSCalcBlast (RSPS As Recordset, RSBlast As Recordset)
                                                                                                                                                                                                                                           Perform all database gets/puts, etc, for BLAST calculation
                                                                                                                                                                                                                                                                                                                                                                                                                                                                           headers of matches
                                                                                                                                                                                                                                                                                                                                                                                                    probe set name.
                                                                                                                                                                                                                                                                                                                                                                                                                               sednence;
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           .Fields("Accession") - MatchAccession(H)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     .Fields("Header") = MatchHeaders(H)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       PSName = RSPS("PSName")
Sequence = RSPS("CreatePS-Sequence")
If Len(Sequence) = 0 Then GoTo UpdateOnly
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        1
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               *Get the probe set name and sequence.
                                                                                                                                                                                                                                                                                                                                                                                                                            Dim Sequence$
Dim MatchAccession$(MaxBLASTHeaders)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               .Fields("Use") - False
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   .Fields("PSName") - PSName
                                                                                                                                                                                                                                                                                                                                                                                                                                                                           Dim MatchHeaders$ (MaxBLASTHeaders)
Dim MatchScores$ (MaxBLASTHeaders)
Dim MatchExpects$ (MaxBLASTHeaders)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              .Fields("Use") = True
End If
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        C Copyright Hewlett-Packard Company, 1998
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             For H = 0 To NumMatches - 1
                                                                                       Attribute VB_Name = "Engine"
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         MatchAccession(H) & "'"
If .NoMatch Then
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         BlastPars.ECutoff Then
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  'Update the results.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      . AddNew
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   Ed1t
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     With RSBlast
                                                                                                                                                                                                                                                                                                                                               On Error GoTo E
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     Dim NumMatchesa
                                                                                                                 Option Explicit
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           Else
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          Dim FindStr$
          End Function
                                                                                                                                                                                                                                                                                                                                                                                                    Dim PSName$
                                                                                                                                                                                                                                                                        'Arguments
                                                                                                                                                                                                                      Function
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             Dia
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Dim PSName$
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       callee's
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 1dea why
                         Notes
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            :
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          Progress. ShowProgress "Updating Probes in Database for Probeset " & PSName, 0,
                                                                                                                                                                                                                                                                                                                                                                                                                                                           NumProbes - CreatePSEqualTM(Seq, CreatePSPars, Probes, Positions, GC)
                                                                                                                                                                                                                                                                                                                                                                  Case "EqualL"
NumProbes = CreatePSEqualL(Seq, CreatePSPars, Probes, Positions, GC)
                                                                                                                                                                                                                                             Progress. ShowProgress "Creating Probeset " & PSName, 0, NumProbes
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  CreatePSPars. AddNewDB RSPS, Accession, PSName, SeqLength, Seq
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                If P - Progress.StopAt = 0 Then Progress.CheckProgress P
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                performed on Sequences. Each of the known calculations can be requested by specifying its name and subname.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            SequalcEngine then retrieves necessary records from the DB, then calls the specified calculation for each probe.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    SequalcEngine acts as the dispatcher for calculations
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           NumProbes * 0 Then Err.Raise ppErrNoProbesCreated
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            RSProbes.Fields("PSName") = PSName
RSProbes.Fields("Sequence") = Probes(P)
RSProbes.Fields("Length") = Len(Probes(P))
RSProbes.Fields("Position") = Positions(P)
RSProbes.Fields("GC") = GC(P)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               RSProbes.Fields("Accession") = Accession
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      E: Debug.Print "Error in SeqCalcCreatePS"
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              'Create new records in the Probes table.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     Err.Raise Err.Number, , Err.Description
PSName - Accession 6 "_PS" & CStr(NumPS)
                                                     'Create temporary store for new probes. ReDim Probes (SeqLength)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          ReDim Preserve Probes (NumProbes)
ReDim Preserve Positions (NumProbes)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        RSSeq.Flelds("Numps") = Numps + 1
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    Public Sub SeqCalcEngine(Calc$)
                                                                                                                                                                                                                                                                                                                                            Select Case CreatePSPars.Method
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             For P = 0 To UBound (Probes) -
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   Rebim Preserve GC (NumProbes)
                                                                                                                           ReDim Positions (SeqLength)
                                                                                                                                                                                                                       'Open the progress bar.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            RSProbes.AddNew
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               'Add a new ProbeSet.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          RSProbes.Update
                                                                                                                                                           ReDim GC (SeqLength)
                                                                                                                                                                                                                                                                                                                'Create the probes.
                                                                                                                                                                                                                                                                                                                                                                                                                                  Case "EqualTM"
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           RSSeq.Update
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   'Clean exit.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 End Select
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 Funct 1on
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       NumProbes
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   Exit Sub
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          Next P
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'Begin a transaction block 'XXXXX -- causes an error when entering this routine for the second time, no
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            .RecordSource = "SELECT * FROM Probes WHERE PSName = '* & PSName &
                                                                                                                                                                                                                                                                                                                      'holds all probes.
'holds all probes in one Probeset
'holds parameters for the current Probeset
                                                                                                                                                                                                                                                                                                                                                                                                                  'current ProbeSet name
'saves number of errors raised by callee's
'save description of errors raised by
                                                                                                                                                                                                          Calling routine is responsible
                                                                                                                                                                                                                                                                                                   holds all selected Sequences

    The parameters used for a particular calculation are
either made available in the optional parameter array
Pars, or retrieved from the class properties.
    This routine loops over all selected Sequences.

                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            'Create the new probeset.
SeqCalcCreatePS RSSelSeqs, RSP, RSPS
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          Set RSSelSeqs = frmMain.datSelSeqs.Recordset
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           'Access all probes in this ProbeSet.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           'Check that some sequences are avallable.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                If Not IsGoodRS (RSSelSeqs) Then Exit Sub
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           Set RSAllProbes - Recordset
                                                                                                                                                                                                          Errors are raised by not handled.
Calc: The calculation to perform.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            Set RSP = frmMain.datP.Recordset
Set RSPS = frmMain.datPS.Recordset
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        *Process all selected sequences.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             PSName = RSPS("PSName")
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         With frmMain.datPonePS
                                                                                                                                                                                                                                                                                                                                  Dim RSP As Recordset
Dim RSAllProbes As Recordset
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       RSSelSeqs.MoveFirst
Do While Not RSSelSeqs.EOF
                                                                                                                                                                                                                                                                                                         Dim RSSelSeqs As Recordset
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                'Map recordset pointers.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 frmMain.dbgProbes.Close
                                                                                                                                                                                                                                                                                                                                                                                            Dim RSPS As Recordset
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  Get PS Name
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             Select Case Calc
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    Case "CreatePs"
                                                                                                                                                                                                                                                                                                                                                                                                                                                       Dim ErrNumber
Dim ErrDescription
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  'Close the grid.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     *Error handling.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      *BeginTrans
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    On Error GoTo E
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Attorney Docket No. 10971 464-1

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RS.Update
RS.MoveNext
                      frmMain.Form_ChangeSequence
                                             frmMain.Form_ChangePSName
                                                                  frmMain.dbgProbes.ReOpen
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                If WhichEnd = "5" Then
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 Do While Not RS.EOF
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          RS.MoveFirst
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             RS.Ed1t
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   Function
                                                                                                                                                                                                                                                                                                                    Function
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         RS.Update
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              Function
Rollback
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     End Sub
                                                                                                                                                                                            End Sub
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            End If
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              Else
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   E13e
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        Attorney Docket No. 10971464-1
                                           'Compute length and position filters.
If LengthFilterpars.Method <>> "None" Then
PSCalcLengthFilter Stallproba; RSPS
PSCalcLengthFilter RsAllproba; IsFilter = LengthFilterpars.AppImmediate
                                                                                                                                                                                            PSCalcPosFilter RSAllProbes, RSPS
PSColumns("Pos Filter"), IsFilter = PosFilterPars.AppImmedlate
                                                                                                                                                                                                                                                                 If GCFilterPars.Method <> "None" Then
PSCalcGCFilter RSAliProbes, RSPS
PSColumns("GC Filter").IsFilter = GCFilterPars.AppImmediate
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              Case "5' Keep"
SeqCalcKeep frmMain.datSelSeqs.Recordset, "5'"
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               SeqCalcTrim frmMain.datSelSeqs.Recordset, "3 **
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         SeqCalcTrim frmMain.datSelSeqs.Recordset, "5""
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      Case "3' Keep"
SeqCalcKeep frmMain.datSelSeqs.Recordset, "3'"
                                                                                                                                                                                                                                                                                                                                                                  PSColumns("PSName").isvisible = True
PSColumns("Sequence").isvisible = True
PSColumns("Length").isvisible = True
PSColumns("Position").isvisible = True
PSColumns("GC").isvisible = True
                                                                                                                                                                     If PosFilterPars.Method <> "None" Then
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        'Handle errors.

E: Debug.Print "Error in SeqCalcEngine."

ErrNumber = Err.Number

ErrDescription = Err.Description
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        7
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             Move to next selected sequence.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           Copyright Hewlett-Packard Company, 1998
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              frmMaln.Form_ChangeSequence
frmMaln.Form_ChangePSName
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     'Reopen the grid.
frmMain.dbgProbes.ReOpen
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   End the transaction
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    RSSelSegs.MoveNext
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    'Update appearances.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     Case "3' Trim"
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               CommitTrans
  End With
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          End Select
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      Progress.Hide
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   'Clean exit.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             Exit Sub
```

```
RS("Sequence") " DNA_SeqKeep(RS("sequence"), TrimPars.ThreePKeep, "3'")
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               RS("Sequence") * DNA_SeqTrim(RS("sequence"), TrimPars.ThreePTrim, "3:")
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             If WhichEnd = "5'" Then
RS("Sequence"), TrimPars.FivePTrim, "5'")
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       RS("Sequence") ** DNA_SeqKeep(RS("sequence"), TrimPars.FlvePKeep, "5'")
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      Private Sub SetField(RS As Recordset, Fields, Value)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    Set all entries for one field in the recordset.
                                                                                                                                                                                                                                                          Private Sub SeqCalcTrim(RS As Recordset, WhichEnds)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          Private Sub SeqCalcKeep(RS As Recordset, WhichEnd$)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           RS("Length") = Len(RS("Sequence"))
RS("Accession") = RS("Accession") & "*"
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  RS("Accession") = RS("Accession") & "*"
                                                                                                                                                                                                                                                                                                                                                                     Trim bases from the 3' or 5' ends.
Progress.Hide
frmMain.MousePointer = vbDefault
Err.Raise ErrNumber, , ErrDescription
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    Keep bases on the 3' or 5' ends.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   RS("Length") = Len(RS("Sequence"))
                                                                                                                                                                                                                                                                                                                                                                                                                              If Not IsGoodRS(RS) Then Exit Sub
RS.Edit
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                If Not IsGoodRS(RS) Then Exit Sub
```

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Attorney Docket No. 10971464-1
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    ASProbes: The probes recordset (calculate dGM for all records).
RSPS: The paramaters recordset (set parameters used in current record).
                                                                                                                                                                                                                                                                                                                                                                                                                                  Progress.ShowProgress "Updating dGH in Database for ProbeSet " & PSName, 0,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                'Store parameters in the database, and update column visibility.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         Private Sub PSCalcdGM(RSProbes As Recordset, RSPS As Recordset)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  Perform all database gets/puts, etc, for dGM calculation.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       'name of current probeset 'number of probes in the recordset 'sequence column from database.'dGM column, to be put to database.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     E: Debug.Print "Error in PscalcdGH"
Err.Raise Err.Number, , Err.Description
End Sub
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              If NumProbes = 0 Then GoTo UpdateOnly
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          7
                                                                                                       'Get the sequences.
GetField RSProbes, "Sequence", Seq
  Create space for database flelds.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      dGHPars.StoreDB RSPS
PSColumns("dGH").Exists = True
PSColumns("dGH").IsVisible = True
                                                                                                                                                                                                                                                                      DNA CalcdGH Seq, dGHPars, dGH
                                                                                                                                                                                                                                                                                                                          RNA_CalcdGH Seq, dGHPars, dGH
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        NumProbes - NumRecords (RSProbes)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          Copyright Hewlett-Packard Company, 1998
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             *Determine the number of probes.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                'Calculate filter
PSCalcdGHFilter RSProbes, RSPS
                           ReDim Seq(0 To NumProbes - 1)
ReDim dGH(0 To NumProbes - 1)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        Putfield RSProbes, "dGH", dGH
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  'Start it up.
PSName = RSProbes("PSName")
                                                                                                                                                                                                                     Select Case dGHPars.DR
                                                                                                                                                                                                                                                                                                                                                                                                         'Update the results.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      On Error GoTo E
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       Dim PsName$
Dim NumProbes&
Dim Seq$()
                                                                                                                                                                                         Calculate dGH
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            UpdateOnly:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 *Arguments
                                                                                                                                                                                                                                          Case "DNA"
                                                                                                                                                                                                                                                                                                                                                  End Select
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      Dim dGM# ()
                                                                                                                                                                                                                                                                                                                                                                                                                                                                NumP robes
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          Funct 1on
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             Exit Sub
                                                                                                                                                                                                                                                                                                 Case
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        Attorney Docket No. 10971464-1
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          Progress. ShowProgress "Calculating dGH for ProbeSet " & PSName, 0, NumProbes
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           RSProbes: The probes recordset (calculate dGH for all records).
RSPS: The parameters recordset (set parameters used in current record).
                                                                                                                                Since this operation can be timeconsuming, the progressbar is updated. So a progressbar has to be posted first!
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           Private Sub PSCalcdGH(RSProbes As Recordset, RSPS As Recordset)
                                                                                                                                                                                                                                                           RS.MoveFirst
Do While Not RS.EOF
If P - Progress.StopAt = 0 Then Progress.CheckProgress P
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       Perform all database gets/puts, etc, for dGH calculation.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           'dGH column, to be put to database.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           'name of current probeset
'number of probes in the recordset
'sequence column from database.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     Private Sub GetField(RS As Recordset, Fields, Values)
Private Sub PutFleld(RS As Recordset, Fields, Value)
                                                                            Put all entries for one field in the recordset.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 Get all entries for one field in the recordset.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        ន
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   If NumProbes - 0 Then GoTo UpdateOnly
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               'Determine the number of probes.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          NumProbes = NumRecords (RSProbes)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     © Copyright Hewlett-Packard Company, 1998
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          Values(P) = RS.Fields(Field)
                                                                                                                                                                                                                                                                                                                                                                     RS.Fields(Field) - Value(P)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      'Start 1t up.
PSName - RSProbes("PSName")
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      RS.Movefirst
Do While Not RS.EOF
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   RS.MoveNext
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        On Error GoTo E
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 Dim NumProbes6
Dim Seq$()
Dim dGH#()
                                                                                                                                                                                                                                                                                                                                                                                                                                                             RS.MoveNext
                                                                                                                                                                                                                                                                                                                                                                                                   RS.Update
                                                                                                                                                                                                                                                                                                                                                                                                                               P = P + 1
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 P = P + 1
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              Dim PSName$
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     Arguments
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   Funct 1on
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               Funct 1on
                                                      Funct 1on
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  D1m P6
P = 0
                                                                                                       Notes
                                                                                                                                                                                                                  Dim Pa
```

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Attorney Docket No. 10971464-1
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          Private Sub PSCalcHomology(RSProbes As Recordset, RSPS As Recordset, RSBlast As
                                            Progress. ShowProgress "Calculating Run for ProbeSet " & PSName, 0, NumProbes
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           RSProbes: The probes recordset (calculate Homology for all records).
RSPS: The parameters recordset (set parameters used in current record).
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         istart it up. Note that the progress bar in this case will show progress over sequences in the database. Since we don't know the total, we'll set the maximum to
                                                                                                                                                                                                                                                                                                                                                                                                                            Progress. ShowProgress "Updating Run in Database for ProbeSet " 6 PSName, 0,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        Perform all database gets/puts, etc, for Homology calculation.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        'Store parameters in the database, and update column visibility.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    'number of probes in the recordset 'sequences of probes
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  'run column, to be put to database
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            'name of current probeset
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              PSColumns ("Run Length").Exists * True
PSColumns ("Run Length").IsVisible = True
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      E: Debug.Print "Error in PSCalckun"
Err.Raise Err.Number, , Err.Description
End Sub
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        56
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      NumProbes = NumRecords (RSProbes)
If NumProbes = 0 Then GoTo UpdateOnly
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           PutField RSProbes, "Run Length", Run
                                                                                                                                                                                                                                    'Get the sequences.
GetField RSProbes, "Position", Pos
                                                                                                          'Create space for database flelds.
ReDim Pos(0 To NumProbes - 1)
ReDim Run(0 To NumProbes - 1)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           'Determine the number of probes.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     © Copyright Hewlett-Packard Company, 1998
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    'Calculate the run filter.
PSCalcRunFilter RSProbes, RSPS
'Start 1t up.
PSName = RSProbes("PSName")
                                                                                                                                                                                                                                                                                                                                                     CalcRun Pos, RunPars, Run
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         RunPars.StoreDB RSPS
                                                                                                                                                                                                                                                                                                                                                                                                          'Update the results.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       On Error GoTo E
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               Dim Homology&()
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                Dim NumProbes£
                                                                                                                                                                                                                                                                                                                    Calculate Run.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  Dim PSName$
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         UpdateOnly:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            Arguments
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           Dim Seq$()
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              Recordset)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    Funct 1on
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        Attorney Docket No. 10971464-1
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        RSPS: The parameters recordset (set parameters used in current record).
rogress.ShowProgress "Calculating dGM for ProbeSet " & PSName, 0, NumProbes
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        Progress. ShowProgress "Updating dGM in Database for ProbeSet " & PSName, 0,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             RSProbes: The probes recordset (calculate Run for all records).
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     'Store parameters in the database, and update column visibility.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 Private Sub PSCalcRun(RSProbes As Recordset, RSPS As Recordset)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       Perform all database gets/puts, etc, for Run calculation.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   'number of probes in the recordset 'position column from database.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              name of current probeset
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               E: Debug.Print "Error in PSCalcdGM."
Err.Raise Err.Number, , Err.Description
End Sub
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     NumProbes = NumRecords (RSProbes)
If NumProbes = 0 Then GoTo UpdateOnly
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              Я
                                                                                                                                                                                  'Get the sequences.
GetField RSProbes, "Sequence", Seq
                                                                    Create space for database fields.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               dGMPars.StoreDB RSPS
PSColumns("dGM").Exists = True
PSColumns("dGM").IsVisible = True
                                                                                                                                                                                                                                                                                                                                                           DNA_calcdGM Seq, dGMPars, dGM
                                                                                                                                                                                                                                                                                                                                                                                                                   RNA_CalcdGM Seq, dGMPars, dGM
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             'Determine the number of probes.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 C Copyright Hewlett-Packard Company, 1998
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              PSCalcdGMFilter RSProbes, RSPS
                                                                                         ReDim Seq(0 To NumProbes - 1)
ReDim dGM(0 To NumProbes - 1)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          Putfield RSProbes, "dGM", dGM
                                                                                                                                                                                                                                                                                                  Select Case dGMPars.DR
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      'Update the results.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   Calculate filter
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         On Error GoTo E
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            Dim NumProbes&
                                                                                                                                                                                                                                                                        'Calculate dGM.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    Dim PSName$
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  UpdateOnly:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          Dim Post ()
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           Arguments
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 Dim Runs ()
                                                                                                                                                                                                                                                                                                                                                                                         Case "RNA"
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   Function
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Attorney Docket No. 10971464-1
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RSPS: The parameters recordset (set parameters used in current record).
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                psName = RsProbes("PsName")
Progress.ShowProgress "Calculating dG (Duplex) for Probeset " & PSName, 0,
                                                                                                                                                                                                                                                                                                                                                                                                                         Progress.ShowProgress "Updating TM in Database for ProbeSet " & PSName, 0,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          'Store parameters in the database, and update column visibility.
TMPars.StoreDB RSPS
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    Private Sub PSCalcdGD(RSProbes As Recordset, RSPS As Recordset)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        Perform all database gets/puts, etc, for dGD calculation.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     'number of probes in the recordset 'sequence column from database. 'dGD column, to be put to database.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                'name of current probeset
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             Err.Raise Err.Number, , Err.Description
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  83
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  If NumProbes = 0 Then GoTo UpdateOnly
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        E: Debug.Print "Error in PSCalcTM"
                                                                                                              GetFleld RSProbes, "Sequence", Seq
                                                                                                                                                                                                                                                              DNA_CalcallTM Seq, TMPars, TM
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       Determine the number of probes.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             NumProbes = NumRecords (RSProbes)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               © Copyright Hewlett-Packard Company, 1998
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      PSColumns("TM").Exists = True
PSColumns("TM").IsVisible = True
                                                                                                                                                                                                                                                                                                                       DR CalcallTM Seq, TMPars, TM
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            Calculate the filter.
PSCalcTMFilter RSProbes, RSPS
ReDim Seq(0 To NumProbes - 1)
                             ReDim TM(0 To NumProbes - 1)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           Putfield RSProbes, "TM", TM
                                                                                                                                                                                                        Select Case TMPars.Duplex
                                                                                                                                                                                                                                                                                                                                                                                                             'Update the results.
                                                                                      'Get the sequences.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       On Error GoTo E
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              Dim NumProbes&
                                                                                                                                                                                                                                                                                         Case "DNA/RNA"
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 Start 1t up.
                                                                                                                                                                                                                                   Case "DNA/DNA"
                                                                                                                                                                          'Calculate TM.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   Dim PSName$
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             UpdateOnly:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         Dim Seq$()
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           *Arguments
                                                                                                                                                                                                                                                                                                                                                        End Select
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        NumP robes
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   'Function
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   Exit Sub
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        Attorney Docket No. 10971464-1
                                                                                                                                                                                                                                                                                                                                                                                                                                        Progress ShowProgress "Updating Homology in Database for ProbeSet " & PSName, 0,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   RSProbes: The probes recordset (calculate TM for all records).
RSPS: The parameters recordset (set parameters used in current record).
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       Progress. ShowProgress "Calculating TM for ProbeSet " & PSName, 0, NumProbes
  '100, and have the called routine do fraction calculations.
PSName = RSProbes("PSName")
Progress.ShowProgress "Calculating Homology for ProbeSet " & PSName, 0, 100
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      'Store parameters in the database, and update column visibility.
HomologyPars.StoreDB RSPS
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   Private Sub PSCalcTM(RSProbes As Recordset, RSPS As Recordset)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         Perform all database gets/puts, etc, for IM calculation.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       'number of probes in the recordset 'sequence column from database.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   'TM column, to be put to database.
                                                                                                                                                                                                                                                                                                                                                     CalcHomology Seq, HomologyPars, Homology, RSBlast, PSName
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            'name of current probeset
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        E: Debug.Print "Error in PSCalcHomology"
Err.Ralse Err.Number, , Err.Description
End Sub
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          PutField RSProbes, "Homology", Homology
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     PSColumns("Homology").Exists = True
PSColumns("Homology").IsVisible = True
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              5
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 If NumProbes = 0 Then GoTo UpdateOnly
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            *Create space for database flelds
                                                                                                                          'Create space for database fields. ReDim Seq(0 To NumProbes - 1)
                                                                                                                                                                                                                                                  'Get the sequences.
GetField RSProbes, "Sequence", Seq
                                                                                                                                                                                      ReDim Homology (0 To NumProbes - 1)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            NumProbes - NumRecords (RSProbes)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           Determine the number of probes.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              Copyright Hewlett-Packard Company, 1998
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     *Calculate the homology filter.
PSCalcHomoFilter RSProbes, RSPS
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        PSName = RSProbes("PSName")
                                                                                                                                                                                                                                                                                                                                                                                                                            Update the results.
                                                                                                                                                                                                                                                                                                                                   Calculate Homology.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      On Error GoTo E
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  Dim PSName$
Dim NumProbes£
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                Start it up.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         UpdateOnly:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           Dim Seq$()
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             Arguments
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  ·Function
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            Ola TM# ()
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  Exit Sub
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Progress.ShowProgress "Updating Clamp in Database for ProbeSet " & PSName, 0,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                - performing the calculation.

2. Filter calculations proceed as above, with the preliminary step of checking the existence of the column to be filtered. If it isn't there, the calculation is done.

3. The Probes grid is closed before DB updates, then opened when they are all over, to reduce screen thrashing.

    checking that the sequences column exists.
    checking that the current parameters set for this calculation are the same as those previously used, and, if not, nulling out all previous calculations.

                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          'Store parameters in the database, and update column visibility. Clampers: StoreDB RSPS PSColumns ("Clamp"). Exists = True PSColumns("Clamp"). Isvisible = True
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              PSCalcEngine acts as the dispatcher for calculations performed on ProbeSets. Each of the known calculations can be requested by specifying its name.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              1. All numeric calculations proceed by
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         E: Debug.Print "Error in PSCalcClamp"
Err.Raise Err.Number, , Err.Description
                                                                                                                                                                                                                                                                                                                                                                                                                        DNA_CalcClamp Seq, ClampPars, Clamp
End Select
                                                                                                                                                                                                                                                                                                                                           DNA_Calcclamp Seq, ClampPars, Clamp
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      ಜ
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 Calc: The calculation to perform.
                      'Create space for database fields.
ReDim Seq(0 To NumProbes - 1)
ReDim Clamp(0 To NumProbes - 1)
                                                                                                                                                  'Get the sequences.
GetField RSProbes, "Sequence", Seq
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    PutField RSProbes, "Clamp", Clamp
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      PSCalcClampFilter RSProbes, RSPS
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      © Copyright Hewlett-Packard Company, 1998
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  Public Sub PSCalcEngine (Calc$)
                                                                                                                                                                                                                                                                           Select Case ClampPars.Duplex Case "DNA/DNA"
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      'Calculate the filter
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              'Update the results.
                                                                                                                                                                                                                                               *Calculate Clamp.
                                                                                                                                                                                                                                                                                                                                                                           Case "DNA/RNA"
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       UpdateOnly:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        *Arguments
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             NumProbes
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              Funct 1on
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 Notes
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                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              Progress.ShowProgress "Updating dG (Duplex) in Database for ProbeSet " & PSName,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                Progress.ShowProgress "Calculating Clamp for ProbeSet " & PSName, 0, NumProbes
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           RSProbes: The probes recordset (calculate Clamp for all records). RSPS: The parameters recordset (set parameters used in current record).
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        Private Sub PSCalcclamp(RSProbes As Recordset, RSPS As Recordset)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          store parameters in the database, and update column visibility.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 'number of probes in the recordset 'sequence column from database. 'Clamp column, to be put to database.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  Perform all database gets/puts, etc, for Clamp calculation.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           'name of current probeset
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       PSColumns (*Duplex dG*).Exists = True
PSColumns (*Duplex dG*).IsVisible = True
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   Err.Raise Err.Number, , Err.Description
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               23
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                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    Putfield RSProbes, "Duplex dG", dGD
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      E: Debug.Print "Error in PSCalcdGD"
                                                                                                                                  'Get the sequences.
GetField RSProbes, "Sequence", Seq
'Create space for database fields. ReDim Seq(0 To NumProbes - 1) ReDim dGD(0 To NumProbes - 1)
                                                                                                                                                                                                                                                                                                                     ggp
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             NumProbes - NumRecords (RSProbes)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   Copyright Hewlett-Packard Company, 1998
                                                                                                                                                                                                                                                                                                                                                                              DR_CalcdGD Seq, dGDPars, dGD
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    *Determine the number of probes.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                'Calculate the filter.
PSCalcdGDFilter RSProbes, RSPS
                                                                                                                                                                                                                                                                                                                  DNA CalcdGD Seq, dGDPars,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          'Start it up.
PSName = RSProbes("PSName")
                                                                                                                                                                                                                                               Select Case dGDPars.Duplex Case "DNA"
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          dGDPars.StoreDB RSPS
                                                                                                                                                                                                                                                                                                                                                                                                                                                                              'Update the results.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 On Error GoTo E
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       Dim NumProbes&
Dim Seq$()
Dim Clamp$()
                                                                                                                                                                                                                            Calculate dGD
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          0. NumProbes
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           Dim PsName$
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    UpdateOnly:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     'Arguments
                                                                                                                                                                                                                                                                                                                                                  Case "RNA"
                                                                                                                                                                                                                                                                                                                                                                                                                 End Select
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        'Function
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Attorney Docket No. 10971464-1

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If Not PSColumns("Tw").Exists Then
If TWPars.Exists(RSPS) And Not TWPars.Validate(RSPS) Then SetField
RSAllProbes, "TM", Null
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   If Not TMFilterPars. Validate (RSPS) Then SetField RSAllProbes, "TM
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              RSAllProbes, "TM", Null
If Not TMFilterPars.Validate(RSPS) Then SetField RSAllProbes, "TM
                                                               'Position the parameter recordset for updates.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            PSCalcdGDFilter RSQProbes, RSPS
End If
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        PSCalcTMF11ter RSQProbes, RSPS
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                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               PSCalcTM RSQProbes, RSPS
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                © Copyright Hewlett-Packard Company, 1998
                                                                                                                                                                                                                                    'Start a transaction block.
                                                                                                                                                                                                                                                                                                                  Choose the operation
                                                                                            With frmMain.datPS
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       Case "dGD Filter"
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        Case "TM Filter"
                                                                                                                                                                                                                                                                                                                                                 Select Case Calc
                                                                                                                                                                                                                                                                BeginTrans
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  End If
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         Case "dGH"
                                                                                                                                                                                                                                                                                                                                                                                                     Case "dGD"
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      Filter", True
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      Filter", True
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             Case "TM"
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                Filter", True
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 Filter", True
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 Filter", True
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        Else
                                                                                                                                                                          End With
        End With
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     Filter",
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                Attorney Docket No. 10971464-1
4. Updates to the database are placed in transaction blocks, so
that they can be cleanly cancelled. This should also improve
performance on network drives.
5. Calculation is always done one ProbeSet at a time, to reduce, whenever
possible, recalculation of values. This could probably be further improved
by only recalculating NULL values (since any non-NULL is guarenteed good by
the above checks).
                                                                                                                                                                                                                                                                                                                                            'holds all selected Probesets
'holds all probes in one Probeset
'holds probes in one Probeset that pass the
                                                                                                                                                                                                                                 Errors are handled by rolling back pending DB transactions, cleaning up the UI, then re-raising the same error for the caller to deal with.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   'current Probeset name 'saves number of errors raised by callee's
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    .RecordSource = "SELECT " FROM Probes WHERE PSName = '" & PSName & "'"
                                                                                                                                                                                                                                                                                                                                                                                                                                                            'holds parameters for the current Probeset 'holds BLAST homologies
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          save description of errors raised by
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              'Check that some ProbeSets are available, with sequence information
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              'Post the initial progress bar.
Progress.ShowProgress "Beginning Calculation: " & Calc, 0, 100
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  'Access probes in this ProbeSet that pass the filters.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           Set RSSelPs = frmMain.datSelPs.Recordset
If Not isGoodRs(RSSelPs) Then Exit sub
If Not PSColumns("Sequence").Exists Then Exit Sub
Set RSBlast = frmEdItBLAST.datBLAST.Recordset
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        .RecordSource - BuildPonePSQuery (PSName)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             'Access all probes in this ProbeSet. With frmMain.datPonePS
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                ౯
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              Set RSAllProbes = .Recordset
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             frmMain.MousePointer = vbHourglass
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               Set RSQProbes = .Recordse
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                Copyright Hewlett-Packard Company, 1998
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    'Process all selected ProbeSets.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           With frmMain.datPOnePSQuery
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      PSName = RSSelPS("PSName")
                                                                                                                                                                                                                                                                                                                                              Dim RSSelPs As Recordset
Dim RSAllprobes As Recordset
                                                                                                                                                                                                                                                                                                                                                                                                     Dim RSQProbes As Recordset filters
                                                                                                                                                                                                                                                                                                                                                                                                                                                            Dim RSBlast As Recordset
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            RSSelPS.MoveFirst
Do While Not RSSelPS.EOF
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             frmMain.dbgProbes.Close
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          Dim ErrDescription
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               Get PS Name.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   'Close the grid.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       .Refresh
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           *Error handling.
On Error GoTo E
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   Dim PSName$
Dim ErrNumber
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          callee's
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If Not PSColumns("dGD").Exists Then If dGDPars.Validate(RSPS) Then SetField RSAllProbes, "dGD", Null If Not dGDF: Validate(RSPS) Then SetField RSAllProbes, "dGD" If Not dGDFilterPars.Validate(RSPS) Then SetField RSAllProbes, "dGD If dGDPars.Exists(RSPS) And Not dGDPars.Validate(RSPS) Then SetField RSAllProbes, "dGD", Null If Not dGDFilterPars.Validate(RSPS) Then SetField RSAllProbes, "dGD .Recordset.FindFirst "PSName = '" 6 PSName 6 "'" Set RSPS = .Recordset

gop. If Not dGDFilterPars.Validate(RSPS) Then SetField RSAllProbes,

If IMPars.Exists(RSPS) And Not IMPars.Validate(RSPS) Then SetField

If Not TMF11terPars.Validate(RSPS) Then SetField RSAllProbes, "TM

If dGHPars, Exists(RSPs) And Not dGHPars.Valldate(RSPs) Then SetField RSAllProbes, "dGH", Null

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If ClampPars.Exists(RSPS) And Not ClampPars.Validate(RSPS) Then SetField
RSAllProbes, "Clamp", Null
If Not ClampFilterPars.Validate(RSPS) Then SetField RSAllProbes, "Clamp
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         Attorney Docket No. 10971 464-1
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   If HomologyPars.Exists(RSPS) And Not HomologyPars.Validate(RSPS) Then
SetField RSAllProbes, "Homology", Null
    If Not HomoFilterPars.Validate(RSPS) Then SetField RSAllProbes,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  If Not PSColumns("Homology").Exists Then
If HomologyPars.Exists(RSPS) And Not HomologyPars.Validate(RSPS)
Then SetField RSAllProbes, "Homology", Null
If Not HomoFilterPars.Validate(RSPS) Then SetField RSAllProbes,
                                                                                                                                                                                                                                           If Not PSColumns("Clamp"). Exists Then
If ClampPars. Exists(RSPS) And Not ClampPars. Validate(RSPS) Then
SetField RSAllProbes, "Clamp", Null
If Not ClampFilterPars. Validate(RSPS) Then SetField RSAllProbes,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            If Not ClampFilterPars.Validate(RSPS) Then SetField RSAllProbes,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            If Not PSColumns ("Position"). Exists Then Exit Sub
If Not PosfilterPars. Validate (RSPS) Then SetField RSAllProbes, "Pos
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            If Not HomoFilterPars.Validate(RSPS) Then SetField RSAllProbes,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              If Not PSColumns("Length").Exists Then Exit Sub
If Not LengthFilterPars.Validate(RSPS) Then SetField RSAllProbes,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              If Not PSColumns("GC").Exists Then Exit Sub
If Not GCFilterPars.Validate(RSPS) Then SetField RSAllProbes,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          PSCalcHomology RSQProbes, RSPS, RSBlast
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                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               "Clamp Filter", True
PscalcclampFilter RSOProbes, RSPS
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      PSCalcHomoFilter RSQProbes, RSPS
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                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           PSCalcPosFilter RSQProbes, RSPS
                                                                                                                                                                                                                                                                                                                                                                                                                                          PSCalcClamp RSQProbes, RSP:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         PSCalcGCFilter RSQProbes, RSPS
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                                                                                                                                                   PSCalcclamp RSQProbes, RSPS
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                PSCalcBlast RSPS, RSBlast
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     Copyright Hewlett-Packard Company, 1998
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                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             Case "Length Filter"
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              "Homology Filter", True
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 Homology Filter", True
                                                                                                                                                                                                                         Case "Clamp Filter"
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               Case "Pos Filter"
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        "Length Filter", True
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  Case "Homology"
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                                                                                                                                                                                                                                                                                                                                                                                                  "Clamp Filter",
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           Case "BLAST
                                                                                                                  Filter", True
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               If Not PSColumns("Run Length").Exists Then
If RunPars.Exists(RSPS) And Not RunPars.Validate(RSPS) Then SetField
RSAllProbes, "Run", True
If Not RunFilterPars.Validate(RSPS) Then SetField RSAllProbes, "Run
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           If Not PSColumns("dGM").Exists Then
If dGMPars.Exists(RSPS) And Not dGMPars.Validate(RSPS) Then SetFleld
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         Attorney Docket No. 10971464-1
                                                                                                                                                                                If Not PSColumns("dCH").Exists Then
If dGHPars.Exists(RSPS) And Not dGHPars.Validate(RSPS) Then SetFleld
                                                                                                                                                                                                                                                      RSAllProbas, "dGH", True
If Not dGHFilterPars.Validate(RSPS) Then SetField RSAllProbes, "dGH
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             RSAllProbes, "dGM", True
If Not dGMFilterPars.Validate(RSPS) Then SetField RSAllProbes, "dGM
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  If Not dGMFilterPars.Validate(RSPS) Then SetField RSAllProbes, "dGM
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                                                                                                                                                                                                                                                                                                                                                                                                                                       If Not dGHFilterPars.Validate(RSPS) Then SetFleld RSAllProbes, "dGH
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                If RunPars.Exists(RSPS) And Not RunPars.Validate(RSPS) Then SetFleld
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            If dGMPars.Exists(RSPS) And Not dGMPars.Validate(RSPS) Then SetField
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If Not RunFilterPars.Validate(RSPS) Then SetField RSAllProbes, "Run
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             RSAllProbes, "dGM", Null
If Not dGMFilterPars.Validate(RSPS) Then SetField RSAllProbes, "dGM
       If Not dGHFilterPars.Validate(RSPS) Then SetField RSAllProbes, "dGH
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           PSCalcRunFilter RSQProbes, RSPS
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                                                                                                                                                                                                                                                                                                                                                                    PSCalcdGH RSQProbes, RSPS
                                                                                PSCalcdGH RSQProbes, RSPS
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                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      Case "dGM Filter"
                                                                                                                                                      Case "dGH Filter"
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   Case "dGM"
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              Case "Run
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      Filter", True
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                                                                                                                                                                                                                                                                                                                                Filter", True
                                                                                                                                                                                                                                                                                                                                                                                                                                                                        Filter", True
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Attorney Docket No. 10971464-1
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            PSName = RSProbes("PSName")
Progress.ShowProgress "Calculating dG (Duplex) Filter for Probeset " & PSName,
                                                                                                                                                                                                                                                                                                                                                                                                                                                         Progress ShowProgress "Updating TM Filter in Database for ProbeSet " 6 PSName,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       RSProbes: The probes recordset (calculate dGD Filter for all records).
RSPS: The parameter recordset (set parameters used in current record).
'Start it up.
PSName = RSProbes("PSName")
Progress.ShowProgress "Calculating TM Filter for ProbeSet " & PSName, 0,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  Private Sub PSCalcdGDFilter(RSProbes As Recordset, RSPS As Recordset)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  Perform all database gets/puts, etc, for dGD filter calculation.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        'Store parameters in the database, and update column visibility.
TMFilterPars.StoreDB RSPS
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                'number of probes in the recordset 'dGD column from database 'dGD Filter column to database
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 PSColumns ("IM Filter") . Isfilter = IMFilterPars. AppImmediate
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        name of current probeset
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        PutField RSProbes, "TM Filter", TMFilter
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 E: Debug.Print "Error in PSCalcTMFilter"
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      Err.Raise Err.Number, , Err.Description
End Sub
                                                                                                                                                                                                                                                                                                                                                                            CalcTMFilter TM, TMFilterPars, TMFilter
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            36
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               NumProbes = NumRecords (RSProbes)
If NumProbes = 0 Then GoTo UpdateOnly
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             *Create space for database fields.
ReDim dGD(0 To NumProbes - 1)
ReDim dGDFilter(0 To NumProbes - 1)
                                                                                                                                                                         ReDim TM(0 To NumProbes - 1)
ReDim TMF11ter(0 To NumProbes - 1)
                                                                                                                                        'Create space for database flelds.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    *Determine the number of probes.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         © Copyright Hewlett-Packard Company, 1998
                                                                                                                                                                                                                                                                                         GetField RSProbes, "IM", TM
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            Dim dGDFilter() As Boolean
                                                                                                                                                                                                                                                                                                                                                                                                                                        'Update the results.
                                                                                                                                                                                                                                                                                                                                                  Calculate TM Filter
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                On Error GoTo E
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           Dim PSName$
Dim NumProbes6
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        Start 1t up.
                                                                                                                                                                                                                                                               'Get the TMs.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             NumP robes
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           0, NumProbes
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      UpdateOnly:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  'Arguments
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          Funct 1on
                                                                                        NumP robes
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    Dim dGD# (
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            Attorney Docket No. 10971464-1
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         RSPS: The parameter recordset (set parameters used in current record).
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         RSProbes: The probes recordset (calculate TM Filter for all records).
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      Private Sub PSCalcIMFilter(RSProbes As Recordset, RSPS As Recordset)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         Perform all database gets/puts, etc, for TM filter calculation.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          'name of current probeset
'number of probes in the recordset
'TH column from database
'TH Filter column to database
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               E: Debug.Print "Error in PSCalcEngine."
ErrNumber = Err.Number
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 If NumProbes = 0 Then GoTo UpdateOnly
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                Err.Raise ErrNumber, , ErrDescription
                                                                                                                                                                                                         'Move to next selected probeset.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               frmMain.MousePointer = vbDefault
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          ErrDescription = Err.Description
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           Determine the number of probes.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         NumProbes = NumRecords (RSProbes)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  Copyright Hewlett-Packard Company, 1998
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    frmMain.MousePointer = vbDefault
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     Dim TM#()
Dim TMFilter() As Boolean
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  frmMain.Form_ChangePSName
frmMain.dbgProbes.ReOpen
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              frmMain. Form ChangePSName
                                                                                                                                                                                                                                                                                                                                                                                                             frmMain.dbgProbes.ReOpen
                                                                                                                      'End the transaction
                                                                                                                                                                                                                                          RSSelPS.MoveNext
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      Update appearances
                                                                                                                                                                                                                                                                                                                                                        'Reopen the grld.
Progress.Hide
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       On Error GoTo E
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       'Handle errors.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              Dim NumProbes6
                                                                                                                                                       CommitTrans
           End If
                                                                    End Select
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        Progress.Hide
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         Clean exit.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                Dim PSName$
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Attorney Docket No. 10971464-1
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                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              RSProbes: The probes recordset (calculate dGH Filter for all records).
RSFS: The parameter recordset (set parameters used in current record).
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         'Start it up.
PSName = RSProbes("PSName")
Progress.ShowProgress "Calculating dGH Filter for ProbeSet " & PSName, 0,
                    Progress.ShowProgress "Updating Clamp Filter in Database for ProbeSet " 6
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             Private Sub PSCalcdGHFilter(RSProbes As Recordset, RSPS As Recordset)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                Perform all database gets/puts, etc, for dGH filter calculation.
                                                                                                                                                                                                                                                                                              PSColumns("Clamp Filter").IsFilter . ClampFilterPars.AppImmedlate
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  'Store parameters in the database, and update column visibility.
dGHFilterPars.StoreDB RSPS
                                                                                                                                                                                                                                 'Store parameters in the database, and update column visibility.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             'number of probes in the recordset 'dGH column from database 'dGH Filter column to database
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  *name of current probeset
                                                                                        PutField RSProbes, "Clamp Filter", ClampFilter
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                                                                                                                                                                                                                                                                                                                                                                                                 E: Debug.Print "Error in PSCalcClampFilter"
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          Putfield RSProbes, "dGH Filter", dGHFilter
                                                                                                                                                                                                                                                                                                                                                                                                                                   Err.Raise Err.Number, , Err.Description
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                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           ReDim dGHFilter(0 To NumProbes - 1)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          'Create space for database fields.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                Copyright Hewlett-Packard Company, 1998
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               *Determine the number of probes.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 NumProbes = NumRecords (RSProbes)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       GetField RSProbes, "dGH", dGH
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            ReDim dGH(0 To NumProbes - 1)
                                                                                                                                                                                                                                                                   ClampFilterPars.StoreDB RSPS
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            Dim dGH#()
Dim dGHFilter() As Boolean
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           'Calculate dGH Filter.
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                                                                PSName, 0, NumProbes
'Update the results.
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                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    Dim NumProbest
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                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               0, NumProbes
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                                                                                                                                                                UpdateOnly:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      *Arguments
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                                                                                                                                                                                                                                 Progress.ShowProgress "Updating dG (Duplex) Filter in Database for ProbeSet " & PSName, 0, NumProbes
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         RSProbes: The probes recordset (calculate Clamp Filter for all records).
RSPS: The parameter recordset (set parameters used in current record).
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            Progress.ShowProgress "Calculating Clamp Filter for ProbeSet " & PSName, 0,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      Private Sub PSCalcClampFilter(RSProbes As Recordset, RSPS As Recordset)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      Perform all database gets/puts, etc, for Clamp filter calculation.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                  'Store parameters in the database, and update column visibility. dGDFilterPars.StoreDB RSPS PSCOlumns("dGD Filter").IsFilter = dGDFilterPars.AppImmedlate
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            'number of probes in the recordset 'Clamp column from database 'Clamp Filter column to database
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    'name of current probeset
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                                                                                                                                 'Calculate dGD Filter.
CalcdGDFilter dGD, dGDFilterPars, dGDFilter
                                                                                                                                                                                                                                                                                                                                    PutField RSProbes, "dGD Filter", dGDFilter
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      E: Debug.Print "Error in PSCalcdGDFilter"
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        Err.Raise Err.Number, , Err.Description
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                                                                       GetField RSProbes, "Duplex dG", dGD
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   'Create space for database flelds.
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GetField RSProbes, "Clamp", Clamp
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             *Determine the number of probes.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               NumProbes - NumRecords (RSProbes)
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                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   Dim ClampFilter() As Boolean
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      'Start 1t up.
PSName = RSProbes("PSName")
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                                        Get the dGDs
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Attorney Docket No. 10971464-1
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                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      RSPIODES: The probes recordset (calculate Run Filter for all records). RSPS: The parameter recordset (set parameters used in current record).
                                                                                                                    RSProbes: The probes recordset (calculate dGM Filter for all records).
                                                                                                                                                   RSPS: The parameter recordset (set parameters used in current record).
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               Progress. ShowProgress "Calculating dGM Filter for ProbeSet " & PSName, 0,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       Private Sub PSCalcRunFilter(RSProbes As Recordset, RSPS As Recordset)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        Perform all database gets/puts, etc, for Run filter calculation.
                                                       Perform all database gets/puts, etc, for dGM filter calculation.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   'Store parameters in the database, and update column visibility.
                                                                                                                                                                                                                                                                                                                            'number of probes in the recordset 'dGM column from database 'dGM Filter column to database
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     dGwFilterPars.StoreDB RSPS
PSColumns("dGM Filter").IsFilter = dGMFilterPars.AppImmedlate
                                                                                                                                                                                                                                                                                             'name of current probeset
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  CalcdGMFilter dGM, dGMFilterPars, dGMFilter
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     0, NumProbes
PutField RSProbes, "dGM Filter", dGMFilter
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 E: Debug.Print "Error in PSCalcdGMFilter"
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       Err.Raise Err.Number, , Err.Description
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               9
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             If NumProbes = 0 Then GoTo UpdateOnly
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  ReDim dGM(0 To NumProbes - 1)
ReDim dGMFilter(0 To NumProbes - 1)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   *Create space for database flelds.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   Copyright Hewlett-Packard Company, 1998
                                                                                                                                                                                                                                                                                                                                                                                                                                                               *Determine the number of probes.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              NumProbes = NumRecords (RSProbes)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     GetField RSProbes, "dGM", dGM
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                PSName = RSProbes("PSName")
                                                                                                                                                                                                                                                                                                                                                                                             Dim dGMFilter() As Boolean
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      *Calculate dGM Filter
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   Update the results.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             On Error GoTo E
                                                                                                                                                                                                                            On Error GoTo E
                                                                                                                                                                                                                                                                                                                            Dim NumProbest
Dim dGM#()
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      Get the dGMs.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  Start 1t up.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     UpdateOnly:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          Arguments
                                                                                                                                                                                                                                                                                             Dim PSName$
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     NumProbes
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         Funct 1on
                           Funct 1on
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         Exit Sub
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      Attorney Docket No. 10971464-1
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 Progress.ShowProgress "Updating GC Filter in Database for ProbeSet " & PSName,
                                                                                                                                                                                                                                                                                                                                                                                             .RSProbes: The probes recordset (calculate GC Filter for all records). RSPS: The parameter recordset (set parameters used in current record).
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       'Start it up.
PSName = RSProbes("PSName")
Progress.ShowProgress "Calculating GC Filter for ProbeSet " & PSName, 0,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    Private Sub PSCalcdGMFilter(RSProbes As Recordset, RSPS As Recordset)
                                                                                                                                                                                                                                   Private Sub PSCalcGCFilter(RSProbes As Recordset, RSPS As Recordset)
                                                                                                                                                                                                                                                                                                                               Perform all database gets/puts, etc, for GC filter calculation.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            'Store parameters in the database, and update column visibility.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               'name of current probeset
'number of probes in the recordset
'GC column from database
'GC Filter column to database
PSColumns("dGH Filter").IsFilter = dGHFilterPars.AppImmedlate
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           GCF11terPars.StoreDB RSPS
PSColumns("GC Filter").IsFilter = GCF11terPars.AppImmediate
                                                                                                                                                                                                                                                                                                                                                                                                                                                                         E: Debug.Print "Error in PSCalcdGHFilter"
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            Putfield RSProbes, "GC Filter", GCFilter
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 E: Debug.Print "Error in PSCalcGCFilter"
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                Err.Raise Err.Number, , Err.Description
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       CalcGCFilter GC, GCFilterPars, GCFilter
                                                                                                                               Err.Raise Err.Number, , Err.Description
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          8
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         If NumProbes = 0 Then GoTo UpdateOnly
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     'Create space for database fields.
ReDim GC(0 To NumProbes - 1)
ReDim GCFilter(0 To NumProbes - 1)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          Copyright Hewlett-Packard Company, 1998
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        'Determine the number of probes.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          NumProbes - NumRecords (RSProbes)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          GetFleld RSProbes, "GC", GC
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      Dim GCFilter() As Boolean
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            Calculate GC Filter.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            'Update the results.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 Error GoTo E
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        Dim NumProbes&
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           Get the GCs.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    NumP robes
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 UpdateOnly:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      Dim PSName$
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              NumP robes
                                                                                                                                                                                                                                                                                                    Funct 1on
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     5
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PSName = RSProbes("PSName")
Progress.ShowProgress "Calculating Homology Filter for ProbeSet " & PSName, 0,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              RSProbes: The probes recordset (calculate length Filter for all records).
RSPS: The parameter recordset (set parameters used in current record).
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              Progress.ShowProgress "Updating Homology Filter in Database for ProbeSet " 6
PSName, 0, NumProbes
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       Progress.ShowProgress "Calculating Length Filter for ProbeSet " & PSName, 0,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          Private Sub PSCalcLengthFilter(RSProbes As Recordset, RSPS As Recordset)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         Perform all database gets/puts, etc, for Length filter calculation.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      HomoFilterPars.StoreDB RSPS
PSColumns("Homology Filter").IsFilter = HomoFilterPars.AppImmediate
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         'name of current probeset
'number of probes in the recordset
'length column from database
'length Filter column to database
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           'Store parameters in the database, and update column visibility.
                                                                                                                                                                                                                                                                                                                                                                                                                'Calculate Homology Filter.
CalcHomoFilter Homology, HomoFilterPars, HomoFilter
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       PutField RSProbes, "Homology Filter", HomoFilter
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 E: Debug.Print *Error in PSCalcHomoFilter*
Err.Raise Err.Number, , Err.Description
End Sub
                                                                                                                                                                                                                                                                                                                             'Get the Homologys.
GetField RSProbes, "Homology", Homology
If NumProbes = 0 Then GoTo UpdateOnly
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      If NumProbes = 0 Then GoTo UpdateOnly
                                                                                                                                                                                                                                        ReDim Homology(0 To NumProbes - 1)
ReDim HomoFilter(0 To NumProbes - 1)
                                                                                                                                                                                                            'Create space for database flelds.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          NumProbes = NumRecords (RSProbes)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            'Determine the number of probes
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  Dim LengthFilter() As Boolean
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           PSName = RSProbes("PSName")
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           'Update the results.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            On Error GoTo E
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        Dim NumProbesa
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      Length& ()
                                                          Start 1t up
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             Start 1t up
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         Dim PSName$
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 UpdateOnly:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           'Arguments
                                                                                                                                                  NumP robes
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        NumProbes
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               Exit Sub
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      D1m
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       RSProbes: The probes recordset (calculate Homology Filter for all records). RSPS: The parameter recordset (set parameters used in current record).
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         Progress.ShowProgress "Updating Run Filter in Database for ProbeSet " & PSName,
                                                                                                                                                                                                                                                                                     'Start it up.
PSName = RSProbes("PSName")
Progress.ShowProgress "Calculating Run Filter for Probeset " & PSName, 0,
NumProbes
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   Perform all database gets/puts, etc, for Homology filter calculation.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             Private Sub PSCalcHomoFilter(RSProbes As Recordset, RSPS As Recordset)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     'Homology Filter column to database
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            'name of current probeset
'number of probes in the recordset
'Homology column from database
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       'Store parameters in the database, and update column visibility.
                                                   'number of probes in the recordset 'Run column from database
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  RunFilterPars.StoreDB RSPS
PSColumns("Run Filter").IsFilter - RunFilterPars.AppImmediate
                                                                                                                'Run Filter column to database
                        name of current probeset
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   CalcRunFilter Run, RunFilterPars, RunFilter

    NumProbes
    PutField RSProbes, "Run Filter", RunFilter

                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   E: Debug.Print "Error in PSCalcRunFilter"
Err.Raise Err.Number, , Err.Description
End Sub
                                                                                                                                                                 'Determine the number of probes.
NumProbes = NumRecords(RSProbes)
If NumProbes = 0 Then GoTo UpdateOnly
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              GetField RSProbes, "Run Length", Run
                                                                                                                                                                                                                                                                                                                                                                                                                                        'Create space for database fields.
ReDim Run(0 To NumProbes - 1)
ReDim RunFilter(0 To NumProbes - 1)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  © Copyright Hewlett-Packard Company, 1998
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               'Determine the number of probes.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              NumProbes - NumRecords (RSProbes)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        Dim NumProbes&
Dim Homology&()
Dim Homofilter() As Boolean
                                                                            Dim Run£()
Dim RunFilter() As Boolean
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     'Calculate Run Filter.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             'Update the results.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     On Error GoTo E
                     Dim PSName$
Dim NumProbest
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                UpdateOnly:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                Dim PSNames
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              'Arguments
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    ·Function
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  Get the
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              Exit Sub
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Attorney Docket No. 10971 464-1

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Attorney Docket No. 10971464-1

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Attorney Docket No. 10971 464-1
                                                                                                                                                                                                                  Progress.ShowProgress "Updating Pos Filter in Database for ProbeSet " 4 PSName,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              Public Sub CalcTMFilter(TM#(), TMFPars As cTMFilterPars, Filter() As Boolean)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             Public Sub CalcdGDFilter(dGD#(), dGDFPars As cdGDFilterPars, Filter() As
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           'The dGD filter can be implemented directly by the generic filter.
CalcGenericFilter dGD, dGDFPars, Filter
End Sub
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               'The TM filter can be implemented directly by the generic filter.
CalcGenericFilter TM, TMFPars, Filter
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 dGD: An array of dGDs dGDFliterPars. dGDFPliterPars. Filter: The return array of filter output values.
                                                                                                                                                                                                                                                                                                                                                                                                             'Store parameters in the database, and update column visibility.
PosFilterPars.StoreDB RSPS
PSColumns("Pos Filter").IsFilter = PosFilterPars.AppImmediate
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               TMFPars: An instance of the parameter class cTMFilterPars.
Filter: The return array of filter output values.
                                                                                                                            CalcPosFilter Pos, Sequength, PosFilterPars, PosFilter
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      Decide which probes pass the dGD filter.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          Decide which probes pass the TM filter.
                                                                                                                                                                                                                                                                                       PutField RSProbes, "Pos Filter", PosFilter
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              E: Debug.Print "Error in PsCalcPosFilter"
Err.Raise Err.Number, , Err.Description
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              'Get the sequence length.
SeqLength = RSPS("CreatePS-SeqLength")
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            4
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         Copyright Hewlett-Packard Company, 1998
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              Attribute VB_Name = "Filters'
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     TM: An array of TMs
                                                                                           *Calculate Pos Filter.
                                                                                                                                                                                       'Update the results.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   Option Explicit
                                                                                                                                                                                                                                                            0, NumProbes
                                                                                                                                                                                                                                                                                                                                                        UpdateOnly:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          'Arguments
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              Arguments
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               Funct 1on
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               Function
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 Boolean)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         Attorney Docket No. 10971464-1
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              ASProbes: The probes recordset (calculate Pos Filter for all records).
RSPS: The parameter recordset (set parameters used in current record).
                                                                                                                                                                                                                                                                                                                                                     Progress.ShowProgress "Updating Length Filter in Database for ProbeSet " 4
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                'Start it up.
PSName = RSProbes("PSName")
Progress.ShowProgress "Calculating Pos Filter for Probeset " & PSName, 0,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          Private Sub PSCalcPosFilter(RSProbes As Recordset, RSPS As Recordset)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      Perform all database gets/puts, etc, for Pos filter calculation.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 LengthFilterPars.StoreDB RSPS
PSColumns("Length Filter").IsFilter - LengthFilterPars.AppImmedlate
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      'number of probes in the recordset
Pos column from datebase
'Pos Filter column to datebase
'Length of the originating sequence.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   'Store parameters in the database, and update column visibility.
                                                                                                                                                                                                                                                         CalcLengthFilter Length, LengthFilterPars, LengthFilter
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            'name of current probeset
                                                                                                                                                                                                                                                                                                                                                                                  PSName, 0, NumProbes
PutField RSProbes, "Length Filter", LengthFilter
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              E: Debug.Print "Error in PSCalcLengthFilter"
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              Err.Raise Err.Number, , Err.Description
'Create space for database fields.
ReDim Length(0 To NumProbes - 1)
ReDim LengthFilter(0 To NumProbes - 1)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         4
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        NumProbes = NumRecords(RSProbes)
If NumProbes = 0 Then GoTo UpdateOnly
                                                                                                                                                          GetField RSProbes, "Length", Length
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               ReDim PosFilter(0 To NumProbes - 1)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              'Create space for database fields.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           'Get the Positions.
GetField RSProbes, "Position", Pos
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       *Determine the number of probes.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         C Copyright Hewlett-Packard Company, 1998
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           ReDim Pos(0 To NumProbes - 1)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       Dim Post()
Dim Posfilter() As Boolean
Dim SeqLength¢
                                                                                                                                                                                                                           'Calculate Length Filter.
                                                                                                                                                                                                                                                                                                                       Update the results.
                                                                                                                            Get the Lengths.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 On Error GoTo E
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            Dim NumProbes6
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  Dim PsName$
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 Arguments
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     UpdateOnly:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      NumP robes
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     'Function
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Attorney Docket No. 10971 464-1
            'If FilterPars Method = "InRange", set all >= min and <= max to true.

If FilterPars Method = "InRange" Then
For T = 0 To NumValues = 1
Filter(T) = ((Values(T) >= Val(FilterPars.Min)) And (Values(T) <= Val(FilterPars.Max))
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             'If method is "Distance", set all elements within distance as true. If FilterPars.Method = "Distance" Then For T = 0 To NumValues - 1
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            Compute distances of Values from target; set up index and filter.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          Filter(T) = Distance(T) <= FilterPars.Distance
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           Then N = NumValues * FilterPars.Percent / 100 - 1
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           If Minvalue > Values(T) Then Minvalue = Values(T) If MaxValue < Values(T) Then MaxValue = Values(T)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    Then N = FilterPars.NBst

If (FilterPars.Method = "Percent") Or

(FilterPars.Method = "PercentLwest") Or

(FilterPars.Method = "PercentHighest")
                                                                                                                                                                                                                                                                                                             For other methods, we have to do more work!
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     If (FilterPars.Method = "NHighest") Or __
(FilterPars.Method = "PercentHighest")
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         Distance(T) = Abs(Values(T) - Target)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               Italierals method = releant) .

If han Target = FilterPars.Target

If (FilterPars.Method = "Nercentlowest") Or

(FilterPars.Method = "Percentlowest")
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          If (FilterPars.Method = "NBest") Or
(FilterPars.Method = "NLowest") Or
(FilterPars.Method = "NHighest")
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 'Choose the target value.

If (FilterPars.Method = "Distance") Or (FilterPars.Method = "NBest") Or (FilterPars.Method = "Percent") ___
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        46
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              'Find the minimum and maximum values.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        Copyright Hewlett-Packard Company, 1998
                                                                                                                                                                                                                                                                                                                                                                                                    ReDim Distance (NumValues - 1)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         Then Target - MinValue
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      Then Target - MaxValue
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            For T = 0 To NumValues - 1
                                                                                                                                                                                                                                                                                                                                                                                                                                 ReDim Index (NumValues - 1)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              For T = 0 To NumValues - 1
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    'Choose the number to set
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   Filter(T) = False
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      Index(T) = T
                                                                                                                                                                                                                                                                                                                                                                       'Size the arrays.
                                                                                                                                                                                                                Exit Sub
                                                                                                                                                                                   Next T
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             Next
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      Next T
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          End If
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           Next T
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        Attorney Docket No. 10971464-1
                                                                                                                                                                                                                                                                             This routine is used to implement the common filter methods. FilterPars is
Public Sub CalcGenericFilter(Values, FilterPars As Object, Filter() As Boolean)
                                                                                                                                                                                                                                                                                                             declared as an object rather than a specific class, and thus needs to have only the members accessed on the execution path.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        absolute difference between values and target
                                                                                                                                                                                                                                                                                                                                                                    FilterPars must have data member Method.

If method is None, no other members are required.

If method is Min, then member Min is required.

If method is Max, then member Max is required.

If method is InRange, then members Max and Min are required.

If method is Distance, then members Target and Distance are required.

If method is NBest, then members Target and Distance are required.

If method is Percent, then members Target and Percent are required.

If method is Percent, then members Target and Percent are required.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  'index array to track sort
'number of values we are working with
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         'Determine the number of probes we are calculating filter for.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          For T = 0 To NumValues - 1 FilterPars.Min))
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 For T = 0 To NumValues - 1
Filter(T) = (Values(T) <= Val(FilterPars.Max))
                                                                                                                                             Values: An array of values to be filtered against FilterPars: The filter parameters Filter: The returned filter settings.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     'target value
'min and max of value
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    'If method is "Max", set all < max to true.
If FilterPars.Method = "Max" Then
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             'If method is "Min", set all >= min to true If FilterPars.Method = "Min" Then
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            'number to set.
                                                                                         Decide which values pass the filter.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         'If method is "None", set all to true.

If FilterPars.Method = "None" Then

For T = 0 To NumValues = 1
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        $
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               · index
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        © Copyright Hewlett-Packard Company, 1998
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      NumValues = UBound(Values) + 1
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             Filter(T) = True
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     Dim Minvalue#, MaxValue#
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        31-Jul-97: PW
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               Dim Indexa()
Dim NumValuesa
Dim Ta
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        Dim Distance#()
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 Exit Sub
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             Exit Sub
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     Dim Target#
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                Next T
                                                                                                                         Arguments
                                                            Funct 1on
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 Next
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 Next
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              End If
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             End If
                                                                                                                                                                                                                                                       Notes
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IS THE THE CHIST

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Attorney Docket No. 10971464-1
Public Sub CalcclampFilter(Clamp*(), ClampFPars As cClampFilterPars, Filter() As
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             Public Sub CalcHomoFilter(Homology&(), HFPars As CHomoFilterPars, Filter() As
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             Public Sub CalcGCFilter(GC#(), GCFPars As cGCFilterPars, Filter() As Boolean)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    Public Sub CalcdGHF11ter(dGH∮(), dGHFPars As cdGHF11terEars, F11ter() As
                                                                                                                                                                             Clamp: An array of Clamps Clamps clampFilterPars. ClampFilterPars. Filter: The return array of filter output values.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       dGHFPars: An instance of the parameter class cdGHFilterPars.
Filter: The return array of filter output values.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   HPPars: An instance of the parameter class cHomoFilterPars.
Filter: The return array of filter output values
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        GCFPars: An instance of the parameter class cGCFilterPars.
Filter: The return array of filter output values.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              Decide which probes pass the homology filter.
                                                                                                                      Decide which probes pass the Clamp Filter
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     Decide which probes pass the dGH filter.
                                                                                                                                                                                                                                                                                                                                                                                                                        CalcGenericFilter Clamp, ClampFPars, Filter
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                CalcGenericFilter Homology, HFPars, Filter
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  Decide which probes pass the GC filter.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        CalcGenericFilter dGH, dGHFPars, Filter
End sub
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           Homology: An array of homologiess
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    8
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            CalcGenericFilter GC, GCFPars, Filter
End Sub
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    Copyright Hewlett-Packard Company, 1998
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               dGH: An array of dGHs
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            GC: An array of GCs
                                                                                                                                                                                                                                                                                                                               31-Jul-97: PW
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            'Arguments
                                                                                                                                                        Arguments
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         Function
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    Function
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     'Funct 1on
                                                                                           Function
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            Boolean)
                                                                                                                                                                                                                                                                                                          'History
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     Boolean)
                                    Boolean)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       Attorney Docket No. 10971464-1
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     'The special method "All" can be handled by using the method Min, with min=1. If RunFPars.Method = "All" Then
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        Public Sub CalcdGMFilter(dGM#(), dGMFPars As cdGMFilterPars, Filter() As
                                                                                                                                                                                                                                                                                                                                                                                            Public Sub CalcRunFilter(Run&(), RunFPars As CRunFilterPars, Filter() As
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    Run: An array of Runs
RunFpars: An instance of the parameter class cRunFilterPars.
Filter: The return array of filter output values.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 dGMFPars: An instance of the parameter class cdGMFilterPars. Filter: The return array of filter output values.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            RunFPars.Method = "Min"
Calcenericfilter Run, RunFPars, Filter
RunFPars.Method = "All"
RunFPars.Min = OldMin
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               Decide which probes pass the dGM filter.
                                                                                              QuickSort Distance, Index, 0, NumValues - 1
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             Decide which probes pass the Run filter
         If N >= NumValues Then N = NumValues - 1
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               'Otherwise, filters are generic.
CalcGenericFilter Run, RunFPars, Filter
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        CalcGenericFilter dGM, dGMFPars, Filter
End Sub
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          4
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          C Copyright Hewlett-Packard Company, 1998
                                                                   Sort on distance from target.
                                                                                                                                                                                For T = 0 To N - 1
Filter(Index(T)) = True
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  OldMin = RunFPars.Min
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            dGM: An array of dGMs
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                RunFPars.Min = 1
                                                                                                                                                        'Set the best.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              Dim OldMing
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             *Arguments
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            *Arguments
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   Function
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  'Funct 1on
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         Boolean)
                                                                                                                                                                                                                                                                                                                                                                                                                           Boolean)
                                                                                                                                                                                                                                                                                                       End Sub
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           End If
                                                                                                                                                                                                                                             Next T
```

DG754674 OB1501

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Attorney Docket No. 10971464-1
                                                                                                                                                       For 1 = 1 To Len(EntrezData)

If (Mid$(EntrezData, 1, 1) = vbLf) Then EntrezData2 = EntrezData2 + vbCr
EntrezData2 = EntrezData2 + Mid$(EntrezData, 1, 1)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             • Attempting to create a new sequence record whose Accession matches a previously inserted sequence causes a DB error.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   Create a new record in the Sequence table, populate from SeqRC
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   SeqRC: A sequence structure, as read from file, etc. RS: The recordset into which the sequence should be loaded
                                                     EntrezData = frmEntrezGrab.Entrez.GetGenBankData(Accession, "")
                                                                                                                                                                                                                                                                                                                     'Write the aquired data out to a file.
GBFileName = frmMain.cdgSeqs.InitDir & "\" & Accession & ".gb"
Open GBFileName For Output As #1
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        Public Sub SeqLoadDB(SeqRC As SeqRecord, RS As Recordset)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             RS.Fields("Header") = SeqRC.Header
RS.Fields("Accession") = SeqRC.Accession
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         RS.Fields("Locus") = SeqRC.Locus
RS.Fields("Length") = SeqRC.Length
RS.Fields("Sequence") = SeqRC.Sequence
RS.Fields("Selected") = True
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        E: Debug.Print "Error in EntrezLoadDB" Err.Raise Err.Number, , Err.Description
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     S
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          If Not KeepFile Then Kill GBFileName
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       'Load the database.
SeqLoadDB ReadGBRecord(GBF11eName),
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            E: Debug.Print "Error in SeqLoadDB"
Rollback
DoEvents
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 RS.Bookmark - RS.LastModified
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  Copyright Hewlett-Packard Company, 1998
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                AllowdbgClick - False
                                                                                                                        Create CRLFs out of LFs.
                                                                                                                                                                                                                                                                                                                                                                                                                         Print #1, EntrezData2
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               AllowdbgClick = True
Exit Sub
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         Remove the file.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               On Error GoTo E
                          'Grab the data.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                RS.Update
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      Committrans
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  BeginTrans
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 DoEvents
                                                                                                                                                                                                                                                           Next 1
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              Attorney Docket No. 10971464-1
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          Public Sub CalcLengthFilter(Length&(), LFPars As cLengthFilterPars, Filter() As
                                                                                          Public Sub CalcPosFilter(Post(), SeqLengthé, FFPars As cPosFilterPars, Filter()
                                                                                                                                                                                                                                                                                      Pos: An array of positions.
Sequength: The length of the sequence from which the probeset was derived.
PPPPATS: An instance of the parameter class cPosFilterPars.
Filter: The return array of filter output values.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    Private Const GBTerminators = "//" 'record terminator in Genbank files Private Const MaxGBFileLength6 = 100000 'maximum allowed GenBank file length
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   Accession: The accession of the required sequence.
RS: The recordset into which this sequence should be loaded.
KeepFile: Controls whether the downloaded file is retained or removed.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        Public Sub EntrezLoadDB (Accessions, RS As Recordset, KeepFile As Boolean)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        instance of the parameter class clengthFilterPars.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      'Genbank sequence file for data.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         'The target for this filter should be the specified end
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               Inength: An array of lengths.
LFPars: An instance of the parameter class cLength
Filter: The return array of filter output values.
                                                                                                                                                                                                                      Decide which probes pass the position filter.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      Decide which probes pass the length filter
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      Grab a sequence using the Entrez CGI.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  CalcGenericFilter Length, LFPars, Filter
End Sub
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     $
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         CalcGenericFilter Pos, PFPars, Filter
End Sub
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 If PFPars.WhichEnd = "5-prime" Then
PFPars.Target = 1
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     Copyright Hewlett-Packard Company, 1998
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       Dim EntrezDatas, EntrezData2$
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            PFPars.Target - SeqLength
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      Attribute VB_Name = "GenBank"
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             On Error GoTo E
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          Option Explicit
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      Dim GBFileName$
                                                                                                                               As Boolean)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        Arguments
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        'Arguments
                                                                                                                                                                                                                                                        *Arguments
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           Funct 1on
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     Funct 1on
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            Boolean)
end Sub
```

```
If GB.Length <> Len (GB.Sequence) Then
MsgBox "Sequence length differs from header specification."
GB.Length = Len (GB.Sequence)
GB.Accession = GB.Accession 6 ***
                                                                                                                                                                                                                                                                                                                                                                                                                                                                         GB. Accession = StrField (GBItem, LineLength, 6)
                                                                                                                                                                                                                                                                                                                               foo = StrFleld(GBItem, LineLength, 2)
GB.Locus = StrFleld(GBItem, LineLength, 10)
GB.Length = StrFleld(GBItem, LineLength, 7)
Case *AccESSION*
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               GB.Sequence = GB.Sequence & Left (GBItem, 10)
GBItem = Right (GBItem, LineLength - 11)
LineLength = LineLength - 11
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                'if keyfield has only two blanks -> subkeyword
Elseff Left(keyfield, 3) <> " "Then
'if keyfield has only 4 blanks -> feature code
Elself Left(Keyfield, 5) <> " Then
                                                                          'extract keyfield, and process item
KeyField = StrField(GBltem, LineLength, 10)
'if keyfield is not numeric -> header
If isNumeric(KeyField) = False Then
'if keyfield occupies first column -> keyword
If Left(KeyField, 1) <> " " Then
                                                                                                                                                                                                                                                                                                                                                                                                                                               foo = StrFleld(GBItem, LineLength,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   GB.Sequence - GB.Sequence & GBltem
End If
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                'it must be a continuation line
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   E: Debug.Print "Error in ReadGBRecord"
Err.Raise Err.Number, , Err.Description
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 GB. Sequence = PackSequence (GB. Sequence)
                                                                                                                                                                                                                                            'process keywords
Select Case KeyField
Case "LOCUS"
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              'sequence has started
Do While LineLength > 10
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          Copyright Hewlett-Packard Company, 1998
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               If LineLength > 0 Then
GoTo breakwhilel
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       'Check sequence length.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            End Select
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       Strip any whitespace.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     ReadGBRecord = GB
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       End If
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     Exit Function
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      End Function
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                breakwhile1:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                close GBFile
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               End If
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    Else
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  End If
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             Attorney Docket No. 10971464-1
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             'check for required fields

If InStr(GBText, "LOCUS") = 0 Then Err.Raise ppErrGBFileFormat, , "No LOCUS field found in GB file."

If InStr(GBText, "ACCESSION") = 0 Then Err.Raise ppErrGBFileFormat, , "No ACCESSION field found in GB file."
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            If INStr(GBText, GBTerminator) = 0 Then Err.Raise ppErrGBFileFormat, , "No
terminator (//) found in GB file."
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      'read in the file as one long string
FileLength = FileLen(FileName)
If FileLength > MaxGBFileLength Then Err.Raise ppErrGBFileLength
GBTaxt = Input (FileLength, #GBFile)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                LineLength = InStr(GBText, vbcrLf) - 1
GBItem = Leff(GBText, LineLength)
GB.Header = GB.Header & GBTtem & vbcrLf
GBTAX = Right(GBText, FileLength - LineLength - 2)
FileLength = FileLength - LineLength - 2)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   'GenBank file
'file length (chars)
'length of current line (chars)
                                                                                                                                                                                              Read a GenBank record from a file into a SegRecord
                                                                                                             Public Function ReadGBRecord (FileNames) As SeqRecord

    Check file is less than MaxGBFileLength
    Check that all required fields are present.

                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          the returned record
                                                                                                                                                                                                                                                    FileName: The filename of the GB record.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            If Left (GBItem, 2) = GBTerminator Then
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             one line
litem keyfield
bit bucket
                         Err.Raise Err.Number, , Err.Description
End Sub
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   entire record
                                                                                                                                                                                                                                                                                                        The GenBank record as a SeqRecord
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        GBFile = FreeFile
Open FileName For Binary As #GBFile
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                C Copyright Hewlett-Packard Company, 1998
                                                                                                                                                                                                                                                                                                                                                               Returned to caller
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 'extract an 1tem
AllowdbgClick - True
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          Dim GB As SeqRecord
                                                                                                                                                                                                                                                                                                                                                                                                                                                                            On Error GoTo E
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                Dim FileLengths
Dim LineLengths
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              process items
Do While True
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           open the file
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             Dim GBitem$
Dim KeyField$
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      Dim GBFile&
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   Dim GBText$
                                                                                                                                                                                                                          *Arguments
                                                                                                                                                                    Function
                                                                                                                                                                                                                                                                              Returns
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       Dim foo$
                                                                                                                                                                                                                                                                                                                                     'Errors:
```

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Line Input #FASTAFile, LineText

If Left (LineText, 1) <> ">" Then Err.Raise ppErrFASTAFormat, , "Header line does not begin w/ >."
                                                                                                                                                                                                                                                                                                                                                                                                LenText = Right(LineText, Len(LineText) - InStr(LineText, "len=") - 3)
If InStr(LenText, "/") <> 0 Then LenText = Left(LenText, InStr(LenText, "/")
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    AccText = Right (LineText, Len(LineText) - InStr(LineText, "gh=") - 2)
If InStr(AccText, "/") <> 0 Then AccText = Left (AccText, InStr(AccText, "/")
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    GBCookseq = GBCookseq & StrField (RawSeq, LineLength, 10) & * *
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           GBCookSeq = GBCookSeq & StrField (RawSeq, LineLength, 10) & "
                                                                                                                      'line processed to find length 'line processed to find accession
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 Format (Format (Bases, "#####"), "@B@B@") 6 " ...
Do While Len(RawSeq) > 10
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   Public Function GBCookseq(ByVal RawSeq$) As String 'Convert raw sequence (no spaces, etc) into more 'palatable form, with spaces every 10 nucleotides and newlines every 60 nucleotides.
                                                                                                one line of the file
                                                                                                                                                                            the returned record
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  E: Debug.Print "Error in ReadFASTARecord"
Err.Raise Err.Number, , Err.Description
End Function
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    'remaining linelength
                                                                                                                                                                                                                                       check it is legal.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       'count the bases
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   SR. Accession - LTrim(RTrim(AccText))
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      GBCookSeq = GBCookSeq & vbCrlf
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  GBCook Seq = GBCook Seq &
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  SR.Length - CLng(LenText)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            Do While LineLength > 60
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       LineLength # Len(RawSeq)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         If Len(RawSeq) <> 0 Then
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        'Extract the accession.
                                                                                                                                                                                                                                  Read the header line,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              Bases = Bases + 60
                                                                                                                                                                                                                                                                                                                                                                         Extract the length.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            ReadFASTAHeader = SR
                                                                                                                                                                         Dim SR As SeqRecord
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              For 1 = 1 To 6
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    Dim LineLengths
                                    On Error GoTo
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 Exit Function
                                                                                             Dim LineText$
                                                                                                                      Dim LenText$
                                                                                                                                                   Dim AccText$
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             D1m 16
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       Dim Basest
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      Bases = 1
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             Next 1
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  Line Input #FASTAFIle, LineText
If Left[LineText, 1] <> ">" Then Err.Raise ppErrFASTAFormat, , "Header line does
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          LineText = Right(LineText, Len(LineText) = InStr(LineText, "len=") = 3)
If InStr(LineText, "/") <> 0 Then LineText = left(LineText, InStr(LineText, "/")
                                                                                                                                                                                                                                                                                                   The FASTA file must already be opened for input, and should be positioned at the start of a FASTA header. It will read an entire sequence, leaving the file positioned on the subsequent header.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   The FASTA header file must already be opened for input, and should be
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     FASTA: The file descriptor for the FASTA header file.
Public Function ReadFASTARecord (FASTAFiles) As SeqRecord
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                Public Function ReadFASTAHeader(FASTAFiles) As SegRecord
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               Read a FASTA header from a file into a SegRecord.
                                                                               Read a FASTA record from a file into a SegRecord
                                                                                                                                       FASTA: The file descriptor for the FASTA file.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                            one line of the file
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   the returned record
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         'Read the header line, check it is legal.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  E: Debug Print "Error in ReadPASTARecord"
Err.Raise Err.Number, , Err.Description
End Function
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               'Read sequence lines till we are done.
Do While (Len(SR.Sequence) <> SR.Length)
Line Input #FASTAFIle, LineText
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    SR. Sequence - SR. Sequence & LineText
                                                                                                                                                                                             The FASTA record as a SegRecord
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           The FASTA record as a SeqRecord
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  at the start of a FASTA header
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            SR.Length = CLng(LineText)
                                                                                                                                                                                                                                                     Returned to caller.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   Returned to caller.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              'Extract the length.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      ReadFASTARecord = SR
                                                                                                                                                                                                                                                                                                                                                                                                                                                                       Dim LineText$
Dim SR As SeqRecord
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        not begin w/ >."
                                                                                                                                                                                                                                                                                                                                                                                                                    Error GoTo E
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              Exit Function
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      Arguments
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       positioned
                                                                                                           *Arguments
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   Funct 1on
                                                     ·Function
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    Errors:
                                                                                                                                                                 Returns
                                                                                                                                                                                                                      'Errors:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 Returns
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             Notes
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wherec = "WHERE ( (PSName = " & PSName & "')"

If PSCOLUMIS ("Describter") .1sFilter Then

If Filter <> " " The Theory of the Company of the Company of the Company of the Company of the Company of the Company of the Company of the Company of the Company of the Company of the Company of the Company of the Company of the Company of the Company of the Company of the Company of the Company of the Company of the Company of the Company of the Company of the Company of the Company of the Company of the Company of the Company of the Company of the Company of the Company of the Company of the Company of the Company of the Company of the Company of the Company of the Company of the Company of the Company of the Company of the Company of the Company of the Company of the Company of the Company of the Company of the Company of the Company of the Company of the Company of the Company of the Company of the Company of the Company of the Company of the Company of the Company of the Company of the Company of the Company of the Company of the Company of the Company of the Company of the Company of the Company of the Company of the Company of the Company of the Company of the Company of the Company of the Company of the Company of the Company of the Company of the Company of the Company of the Company of the Company of the Company of the Company of the Company of the Company of the Company of the Company of the Company of the Company of the Company of the Company of the Company of the Company of the Company of the Company of the Company of the Company of the Company of the Company of the Company of the Company of the Company of the Company of the Company of the Company of the Company of the Company of the Company of the Company of the Company of the Company of the Company of the Company of the Company of the Company of the Company of the Company of the Company of the Company of the Company of the Company of the Company of the Company of the Company of the Company of the Company of the Company of the Company of the Company of the 
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              FilterC = FilterC & " AND ((" & PSColumns(F).Name & ") = True)"
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      named in the Columns table) are returned, to match the layout of the Probes grid. The visibility of various columns is controlled by the column Visible properties.

3. The currently set filters are used as a further selection on the probes returned.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    in an empty recordset.
2. All flelds corresponding to a column (that is, all flelds
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 Build an SQL Query to select Probes from the Probes table
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             FilterC = "([" & PSColumns(F).Name & "] = True)"
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 1. Accession or PSName - <none> is allowed, and results
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   WhereC = WhereC & " AND ([User Filter] <> 0]) ) "
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           WhereC = WhereC & " AND ( " & FilterC & " ) ) "
                                                                                                                                                                                                                                                                                                                  'set up the the from clause -- always the Probes table.
FromC = "FROM Probes"
                                                                                 'For C = 1 To Pscolumns.count
If C <> 1 Then SelectC = SelectC & ", "
selectC = SelectC & "(" & Pscolumns(C).Name & "]
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         'Put it all together,
BuildPonepSQuery = SelectC & FromC & WhereC & ";"
'Set up the select clause -- return all columns.
SelectC = "SELECT * "
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     PSName: The ProbeSet name to search on
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      'Set up the where clause

For F = 1 To PSColumns.Count

If (PSColumns(F).IsFilter = True) Then

If FilterC = "" Then
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  search on
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  Public Function BuildProbesQuery()
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       Accession: The accession to
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  WhereC = WhereC & . ) *
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  AND " & FilterC & ") ) "
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  If FilterC <> "" Then
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      End If
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          Else
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                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          End If
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        Funct 1on
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              Else
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                                                                                                                                                                                                                                                          Next C
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                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            Next F
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               Else
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        RTFGBCookseq = RTFGBCookseq & StrField(Rawseq, LineLength, 10) & " "
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             RTFGBCookseq = RTFGBCookseq & StrField(RawSeq, LineLength, 10) & " "
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               2. All flelds corresponding to a column (that is, all flelds named in the Columns table) are returned, to match the layout of the Probes grid. The visibility of various columns is controlled by the column Visible properties.

3. The currently sat filters are used as a further selection on the probes returned.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 Build an SQL Query to select Probes from the Probes table that pass all filters, and come from the named ProbeSet.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        RTFGBCookSeq = RTFGBCookSeq 6 Format (Format (Format (Bases, "#####"), "@@@@@") 6 " Do While Len(RawSeq) > 10
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             RTFGBCookseq = RTFGBCookseq 6 Format (Format (Bases, "#####"), "@@@@@") 6 " "
                                                                                                                                                                                                                                                               Public Function RIFGBCookSeq(yVal RawSeq$) As String
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           Dim selectcs, Fromcs, Filtercs, Wherecs, OrderbyCs
Dim Cs, Fs
                                                                                                                                                                                                                                                                                                             'Convert raw sequence (no spaces, etc) into more 'palatable form, with spaces every 10 nucleotides
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              PSName: The ProbeSet name to search on.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                Accession: The accession to search on.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    count the bases remaining linelength
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             TFGBCookSeq = RTFGBCookSeq & RawSeq
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 Public Function BuildPonePSQuery(PSName)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    RTFGBCookSeg = RTFGBCookSeg & "\par"
                                                                                                                                                                                                                                                                                                                                                                                                                   and newlines every 60 nucleotides.
                                                                            GBCookSeq = GBCookSeq & RawSeq
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             Attribute VB_Name = "SQL"
Option Explicit
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          If Len (RawSeq) <> 0 Then
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            Bases = 1
Do While LineLength > 60
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        LineLength - Len(RawSeq)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         Bases - Bases + 60
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       Dim LineLength&
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              End Function
                                                                                                                                                                        End Function
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       Arguments
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 Dim 16
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              Dim Basesa
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        Funct 1on
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                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  Statistics.Range = Max - Min
Statistics.Average = Total / Statistics.Count
Statistics.Std = Sqr((Total2 / Statistics.Count) - Statistics.Average *
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       Statistics.Ten = Vector(L + (Statistics.Count - 1) * 0.1)
Statistics.Median = Vector(L + (Statistics.Count - 1) * 0.5)
Statistics.Ninety = Vector(L + (Statistics.Count - 1) * 0.9)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      'Take a pass through to get min, max, total, total^2, index.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   Statistics.Count = UBound(Vector) - LBound(Vector) + 1 ReDim Index(L To U)
                                                                                                                                                                                                                                                                                                                                                                         Public Function Statistics (Vector#()) As StatRecord
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             Total = Total + Vector(1)
Total2 = Total2 + Vector(1) * Vector(1)
Total2 = Total2 + Vector(1) * Vector(1)
If Vector(1) > Max Then Max = Vector(1)
If Vector(1) < Min Then Min = Vector(1)
                                                                                                                                                                                                                                                                                                                                                                                                                                                         Calculate all statistics of a vector.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             8
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       'Set min, max, range, average, std.
Statistics.Min = Min
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          © Copyright Hewlett-Packard Company, 1998
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         Dim Min#, Max#, Total#, Total2#
Dim Index#()
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  QuickSort Vector, Index, L, U
Option Explicit
Public Type StatRecord
                                                                                                                                                                                                                                       Average As Double
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 Statistics.Max = Max
                                                                                                                                                                Ninety As Double
                                                                                                                                 Median As Double
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        Statistics. Average)
                                                                                                                                                                                                                 Range As Double
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           L = LBound (Vector)
U = UBound (Vector)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     'Get array bounds.
                                                       Count As Long
                                                                             Min As Double
Ten As Double
                                                                                                                                                                                           Max As Double
                                                                                                                                                                                                                                                                        Std As Double
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       Min = Vector(L)

Max = Vector(L)

For 1 = L To U
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       Index(1) = 1
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           'Set the count.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            Percentiles.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      Dim Le, Ue
                                                                                                                                                                                                                                                                                                                                                                                                                                      Funct 1on
                                                                                                                                                                                                                                                                                                    End Type
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         Next 1
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  Attorney Docket No. 10971464-1
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            If PSColumns("User Filter").IsFilter Then
   If FilterC <> "" Then
        whereC = WhereC & " AND ( ([User Filter] = 2) OR ( ([User Filter] = 1)
AND " & FilterC & ") ) ) "
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          (Col.1ssort = "Ascending") Then OrderByC = "[" & Col.Name & "] ASC " (Col.1ssort = "Descending") Then OrderByC = "(" & Col.Name & ") DESC "
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          Orderbyc - "ORDER BY Probes. Accession, Probes. PSName, " & Orderbyc
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   FilterC = FilterC & " AND ({" & Col.Name & "] = True)"
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        BulldProbesQuery = SelectC & FromC & WhereC & OrderByC & ";"
                                                                                                                                                                                                   'For C = 1 To PSColumns.Count
If C <> 1 Then SelectC = SelectC & ", "
SelectC = SelectC & "Erobes.[" & PSColumns(C).Name & "]
                                                                                                                                                                                                                                                                                                                                  'set up the the from clause -- from the SelectProbes query. From = "FROM SelectProbes ^{\rm L}
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    WhereC = WhereC & " AND ({User Filter} <> 0]) ) "
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      If FilterC <> "" Then WhereC & " AND ( " & FilterC & " ) ) "
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 'Set up the orderby clause.
'Default to sorting by position, then check columns.
OrderByC = "Position"
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    FilterC = "([" & Col.Name & "] = True)"
                                   Dim Selectcs, Fromcs, Filtercs, Wherecs, OrderBycs Dim Ct, Ft Dim Col As ccolumn
                                                                                                                                                  'set up the select clause -- return all columns.
                                                                                                                                                                                                                                                                                                                                                                                                             'set up the where clause -- filter the rows
'For F = 1 To PSColumns.count
For Each Col In PSColumns
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             5
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 Wherec = "WHERE ( (Selected = True)"
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             If (Col.IsFilter = True) Then
If Filterc = "" Then
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             Copyright Hewlett-Packard Company, 1998
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    Wherec = Wherec 6 " ) "
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          Attribute VB Name = "Stats"
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            For Each Col In PSColumns
                                                                                                                                                                                   SelectC = "SELECT * "
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          Put it all together
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 End If
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     E13e
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          End Function
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               End If
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                Else
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      Else
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              Next Col
                                                                                                                                                                                                                                                                                      Next C
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   Else
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Attorney Docket No. 10971464-1
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    For I = LBound(G, 1) To UBound(G, 1)

For J = LBound(G, 2) To UBound(G, 2)

For K = LBound(G, 3) To UBound(G, 3)

For L = LBound(G, 4) To UBound(G, 4)

S(I, J, K, L) = (G(I, J, K, L) - H(I, J, K, L)) / T
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     1. The reason for this functions existence is that 2uker provides G at 37, and H. S is needed to change temperatures.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    Calculate S given G, H, and T, for all elements of array
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 G: The given free energies.H: The given enthalpies.T: The temperature at which free energy was calculated.S: The returned entropies.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        Private Sub CalcSFromGH(Dims&, G*(), H*(), ByVal T*, S*())
                                                                                                                                                                                                                         Private RNAHP StackH# (0 To 3, 0 To 3, 0 To 3, 0 To 3)
Private RNAHP TStackH# (0 To 3, 0 To 3, 0 To 3, 0 To 3)
Private RNAHP DangleH# (0 To 1, 0 To 3, 0 To 3, 0 To 3)
Private RNAHP LoopH# (0 To 2, 0 To NumCukeLoops - 1)
Private RNAHP TLOOPH# (0 To NumTetraLoops - 1)
                                                                                                                                                                                                                                                                                                                                                                                                                                                         Private RNAHF Tstacks#(0 To 3, 0 To 3, 0 To 3, 0 To 3)
Private RNAHF Tstacks#(0 To 3, 0 To 3, 0 To 3, 0 To 3)
Private RNAHF Danqles#(0 To 1, 0 To 3, 0 To 3, 0 To 3)
Private RNAHF Loops#(0 To 2, 0 To NumCukerLoops - 1)
Private RNAHF Troops#(0 To NumTetraloops - 1)
Private RNAHP_StackG#(0 To 3, 0 To 3, 0 To 3, 0 To 3)
Private RNAHP_DangleG#(0 To 1, 0 To 3, 0 To 3, 0 To 3)
Private RNAHP_DangleG#(0 To 1, 0 To 3, 0 To 3, 0 To 3)
Private RNAHP_LopG#(0 To 2, 0 To NumCakeLoops - 1)
Private RNAHP_TLoopG#(0 To NumTetraloops - 1)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      Dims: The number of dimensions of G, H, and S.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            For J = LBound(G, 2) To UBound(G, 2)
S(I, J) = (G(I, J) - H(I, J)) / T
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  For I = LBound(G, 1) To UBound(G, 1)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      જ
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     For I = LBound (G) To UBound (G)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      S(I) = (G(I) - H(I)) / T
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          Copyright Hewlett-Packard Company, 1998
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     Next L
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          Next K
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              Dim Is, Je, Ke, Le
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             Select Case Dims
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          Next J
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 Next J
                                                                                                                                                                                                        'RNA enthalpy
                                                                                                                                                                                                                                                                                                                                                                                                                                  RNA entropy.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       Next I
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  Next I
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            *Arguments
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             'Function
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    Notes
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        Case 2
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              Case ]
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   Case
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          Attorney Docket No. 10971464-1
                                                                                                                                                                                                                                                                                                                                                                                                                                                                      'DNA/RNA nearest neighbor entropies.'DNA/DNA intlation entropies.'DNA/DNA solf-symmetry entropies.'DNA/DNA TA end enthalpy.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     'DNA/RNA nearest neighbor entropies.
'DNA/RNA initiation
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            'The special case loop lengths.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   "Minumum allowed loop.
"Number of special tetraloops.
'Indices of tetraloops.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  *Number of special case loops.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         'Zuker provides H and G # 37. So on load, calculate S, then recalculate G as
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           *Constants for large-loop
                                                                                                                                                                                                                                                                                                                                                                             constant, cal/mol/deg
                                                                                                                                                                                                                                                There is no good reason to have two sets of thermodynamic parameters, other than history...
                                                                                                                                                                                                                                                                                                                                                                                                              'DNA/DNA nearest neighbor
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          'DNA/RNA nearest neighbor
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      *DNA entropy.

Private DNAHP_StackS#(0 To 3, 0 To 3, 0 To 3, 0 To 3)

Private DNAHP_TstackS#(0 To 3, 0 To 3, 0 To 3, 0 To 3)

Private DNAHP_DangleS#(0 To 1, 0 To 3, 0 To 3, 0 To 3)

Private DNAHP_LoopS#(0 To 2, 0 To NumzukerLoops - 1)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       Private DNAHP_StackG#(0 To 3, 0 To 3, 0 To 3, 0 To 3)
Private DNAHP_TStackG#(0 To 3, 0 To 3, 0 To 3, 0 To 3)
Private DNAHP_DangleG#(0 To 1, 0 To 3, 0 To 3, 0 To 3)
Private DNAHP_LoopG#(0 To 2, 0 To NumCukerLoops - 1)
Private DNAHP_TLoopG#(0 To NumTetraLoops - 1)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    Private DNAHP StackH#(0 To 3, 0 To 3, 0 To 3, 0 To 3)
Private DNAHP TStackH#(0 To 3, 0 To 3, 0 To 3, 0 To 3)
Private DNAHP DangleH#(0 To 1, 0 To 3, 0 To 3, 0 To 3)
Private DNAHP LoopH#(0 To 2, 0 To NumZukerLoops - 1)
Private DNAHP TLoopH#(0 To NumTetraLoops - 1)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    Private DNAHP_TLoops# (0 To NumTetraLoops - 1)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   =
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       Private Const NumTetraLoops6 * 8
Private ZTetraLoops6(0 To NumTetraLoops - 1)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            Private 2Looplengths# (0 To Num2ukerLoops
Private Const DNA_ZMiscLoop# = 1.079
Private Const RNA_ZMiscLoop# = 1.079
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      Parameters used by hairpin calculations.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                8
                                                                                                                                                                                                                                                                                                                                                'Parameters used by TM calculations.
Public Const RGas# = 1.987
Private DNA_DuplexH#(0 To 3, 0 To 3)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          Private DR_DuplexH#(0 To 3, 0 To 3)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     Private DR DuplexS#(0 To 3, 0 To 3)
Private DR_InitH#, DR_InitS#
                                                                                                                                                                                                                                                                                                                                                                                                                                                                         Private DNA Duplexs#(0 To 3, 0 To .
Private DNA_initGCs#, DNA_initATs#
Private DNA_selfs#
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  Private Const NumZukerLoops& = 30
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                Copyright Hewlett-Packard Company, 1998
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                Private Const ZMinLoops = 4
                                                                                                                                                          Attribute VB_Name = "Thermo
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                Private DNA_EndTAH
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 enthaply/entropy.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          DNA free energy.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          'RNA free energy
                                                                                                                                                                                               Option Explicit
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   DNA enthalpy.
                                                              End Function
                                                                                                                                                                                                                                                                                                                                                                                                                                                      enthalples.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 enthalpies.
```

```
RNA_BestHairpin = 1000#
                                                                                                                                                                                                                                                                 Dim ThisHairpin#
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                Dim Energies#()
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   End Function
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              Next TP
                                                                End Function
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  End Select
Next TP
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         *Arguments
                                                                                                                                                                                                   *Arguments
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             Funct ion
                                                                                                                                                       Funct 1on
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       Next FP
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     Case 0
                       Next FP
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           Case 7
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         Case
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 Case
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        For I = LBound(G, 1) To UBound(G, 1)

For J = LBound(G, 2) To UBound(G, 3)

For K = LBound(G, 3) To UBound(G, 3)

For L = LBound(G, 4) To UBound(G, 4)

G(I, J, K, L) = 0.1 * CDbl(Cint(10) * (H(I, J, K, L) + S(I,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     For TP = FP + ZMinLoop + 1 To NumBases - 1
ThisHairpin = DNA Hairpin(Seq, FP, TP)
If ThisHairpin < DNA_BestHairpin Then DNA_BestHairpin = ThisHairpin
                                                                                                                                                                                                                                                                                                                                                                                                                                                                         For I = LBound(G, 1) To UBound(G, 1)

For J = LBound(G, 2) To UBound(G, 2)

G(I, J) = 0.1 * CDbl(CInt(10# * (H(I, J) + S(I, J) * T)))
                                                                                                                Calculate G given H, S, and T, for all elements of array G.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   Compute the most stable hairpin for the given sequence
                                                Private Sub CalcGFromHS(Dimst, H#(), S#(), ByVal T#, G#())
                                                                                                                                                                                                                                                                                                                                                                                      For I = LBound(G) To UBound(G)
G(I) = 0.1 * CDb1(CInt(10# * (H(I) + S(I) * T)))
                                                                                                                                                                                        H: The given enthalples.S: The given entropies.T: The temperature at which free energy is required.S: The returned free energies.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               Seq: The numerical representation of the sequence.
                                                                                                                                                                 Dims: The number of dimensions of G, H, and S.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       Private Function DNA_BestHairpin# (Seq*())
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             NumBases = UBound (Seq) - LBound (Seq) + 1
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       For FP = 0 To NumBases - ZMinLoop - 2
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 © Copyright Hewlett-Packard Company, 1998
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      DNA_BestHairpin = 1000#
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    Next L
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           Next K
                                                                                                                                                                                                                                                                                                    Dim I&, J&, K&, L&
T = T + 273.15
Select Case Dims
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 Dim ThisHairpin#
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     Next J
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  Next J
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           Numbases
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  Dim FP4, TP4
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      * Arguments
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    Next I
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           End Select
                                                                                                                                                                                                                                                                                                                                                                                                                                         Next I
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            Next I
                                                                                                                                                *Arguments
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        ·Function
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 Case 4
                                                                                                                                                                                                                                                                                                                                                                         case 1
                                                                                                                                                                                                                                                                                                                                                                                                                                                                  Case 2
```

For FP = 0 To Numbases - 2MinLoop - 2
For TP = FP + 2MinLoop + 1 To Numbases - 1
ThisHairpin = RNA_Hairpin(Seq, FP, TP)
If ThisHairpin < RNA_BestHairpin Then RNA_BestHairpin = ThisHairpin Private Function RNA_Hairpin*(Seq*(), ByVal FP&, ByVal TP&) Seq: The sequence, in numeric representation. FP: The index of the 5'-end base that closes the loop. TP: The index of the 3' end base that closes the loop. Compute the most stable hairpin for the given sequence. (Seq(TP) <> 1 And Seq(TP) <> 3) Then Exit Function If (Seq(TP) <> 0 And Seq(TP) <> 2) Then Exit Function Seq: The numerical representation of the sequence. Calculate the free energy of a RNA hairpin 'Check whether this pair can close the loop. RNA Hairpin = 1000# Select Case Seq(FP) NumBases = UBound(Seq) - LBound(Seq) + 1 Private Function RNA_BestHairpin#(Seq*()) NumBases = UBound (Seq) - LBound (Seq) + 1 If Seq (TP) <> 3 Then Exit Function If Seq(TP) <> 2 Then Exit Function 'setup to store all possible energies Dim NumBases, StemLength, St *Calculate sequence size.

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Dim NumBases, StemLength, S&
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       *Loop over stem members
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          FP = FP - 1
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               TP = TP + 1
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              Case 0
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        case 2
                                                                                                                                                                                                                                                                         Dim Energies#()
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    Do While True
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           Case
End Function
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                End Select
                                                                                                                                                            Arguments
                                                                                                             'Function
                                                                                                                                                                                                                                                                                                                                                                                                                   Case 0
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          Case
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     Case
                                                                                                                                                                                                                                                                                                                                                                                                                                                                Case
                                                                                                                 , add the stacking interaction of the first mismatch in the loop. Energies (0) = Energies (0) + RNAHP_TStackG(Seq(FP), Seq(FP + 1), Seq(TP), Seq(TP
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           'Add dangles, if they exist.

If (FP >= 0) Then Energies(S) = Energies(S) + RNAHP_DangleG(1, Seq(TP - 1),

Seq(FP + 1), Seq(FP)

If (TP < NumBases) Then Energies(S) = Energies(S) + RNAHP_DangleG(0, Seq(TP -
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    'Record the energy if the helix breaks here. Energies(S) = Energies(S) + RNAHP_DangleG(I, Seq(TP - 1), Seq(FP + 1),
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            'Add the next stacking term
Energies(5 + 1) = Energies(5) + RNAHP_StackG(Seq(FP), Seq(FP + 1), Seq(TP), Seq(TP - 1))
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       If Energies(S) < RNA_Hairpin Then RNA_Hairpin = Energies(S)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         RNAHP_DangleG(0, Seq(TP - 1), Seq(TP), Seq(FP + 1))
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  (Seq(TP) <> 1 And Seq(TP) <> 3) Then Exit Do
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          If (Seq(TP) <> 0 And Seq(TP) <> 2) Then Exit Do
                                                                                                                                                                                                                                                                                                                                                       'Check that there are still bases to process.
If (FP >= 0) And (TP < NumBases) Then
                                                                                                                                                                                                                                                                                                                                                                                                                            "Check that we are still in the helix.
         ReDim Energies (UBound (Seq) - LBound (Seq) + 1)
                                                    'Start the energy calculations with the loop.
Energies(0) = RNAHP_LoopG(2, TP - FP - 2)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            Seq(TP) <> 3 Then Exit Do
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         If Seq(TP) <> 2 Then Exit Do
                                                                                                                                                                                                                                                                                                                                                                                                                                                         Select Case Seq(FP)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       1), Seq(TP), Seq(FP + 1))
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              'Find the minimum energy.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    StemLength = S
For S = 0 To StemLength
                                                                                                                                                                                                                       'Loop over stem members
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 RNA_Hairpin = 1000#
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           End Select
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  S = S + 1
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            Exit Do
                                                                                                                                                                                                                                                                                          FP = FP - 1
TP = TP + 1
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                Case 0
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   Case
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           Case
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      Case
                                                                                                                                                                                                                                                                       Do While True
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               End If
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          Else
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             Next S
```

```
'Add the stacking interaction of the first mismatch in the loop.
Energies(0) = Energies(0) + DNAHP_TStackG(Seq(FP), Seq(FP + 1), Seq(TP), Seq(TP
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           (Seq(TP) <> 1 And Seq(TP) <> 3) Then Exit Do
Private Function DNA_Hairpin#(Seq#(), ByVal FP&, ByVal TP&)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              If (Seq(TP) <> 0 And Seq(TP) <> 2) Then Exit Function
                                                                                                                                                               Seq: The sequence, in numeric representation. FP: The index of the 5'-end base that closes the loop. TP: The index of the 3' end base that closes the loop.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          (seq(TP) <> 1 And Seq(TP) <> 3) Then Exit Function
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   'Check that there are still bases to process.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           'Check that we are still in the helix.
Select Case Seq(FP)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           'Setup to store all possible energies.
ReDim Energies(UBound(Seq) - LBound(Seq) + 1)
                                                                                                    Calculate the free energy of a DNA hairpin
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  'Start the energy calculations with the loop. Energies(0) = DNAHP_LoopG(2, TP - FP - 2)
                                                                                                                                                                                                                                                                                                                                                                                      'Check whether this pair can close the loop.

DNA Hairpin = 1000#

Select case Seq(FP)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             If (FP >= 0) And (TP < NumBases) Then
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            If Seq(TP) <> 3 Then Exit Do
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          If Seq(TP) <> 2 Then Exit Do
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         •Calculate sequence size.
NumBases = UBound(Seq) - LBound(Seq) + 1
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     If Seq(TP) <> 3 Then Exit Function
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  If Seq(TP) <> 2 Then Exit Function
```

```
'Record the energy if the helix breaks here. Energies(S) \blacksquare Energies(S) \blacksquare Energies(S) \blacksquare Energies(S) \blacksquare
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        (TP < NumBases) Then Energies(S) = Energies(S) + DNAHP_DangleG(0, Seq(TP
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        These parameters are from SantaLucia et al. Biochemistry, v. 35, pp 3555.
as found by PkW. DuplexH is in kcal/mol, DuplexS in cal/mol/deg K.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 (FP >= 0) Then Energies(S) = Energies(S) + DNAHP_DangleG(1, Seq(TP = 1), q(FP + 1), Seq(FP)
                                                                                                                      'Add the next stacking term \frac{1}{2} + DNAHP_stackG(Seq(FP), Seq(FP + 1), Energies(S + 1) = Energies(F) + DNAHP_stackG(Seq(FP), Seq(FP + 1),
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              arrays for ease-of-use. Thus this routine must be called by hain to initialize everything.

2. The nearest-neighbor parameters are accessed by indexing by the first then second base, with A->0, C->1, G->2, TU->3. The hairpin parameters are loaded from files by this routine, and the entropy matrices filled in. After this operation, the free energy matrices may be over-written at any time.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                Initialize all the fixed parameters used for Thermo calculations
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                Steminath = S
For S = 0 To Steminath
If Energies(S) < DNA_Hairpin Then DNA_Hairpin = Energies(S)
                                                                                                                                                                                                                                                                                                               DNAHP_DangleG(0, Seq(TP - 1), Seq(TP), Seq(FP + 1))
                        2
Case 3
If (Seq(TP) <> 0 And Seq(TP) <> 2) Then Exit
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 'files opened for Zuker parameters
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   Initialize the DNA Duplex enthalpy and entropy.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      'bitbucket.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            'Add dangles, if they exist
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        Public Sub InitThermoPars()
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               1), Seq(TP), Seq(FP + 1))
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            'Find the minimum energy
                                                                                                                                                                                Seq(TP), Seq(TP - 1))
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       DNA_Hairpin = 1000#
                                                               End Select
                                                                                                                                                                                                                                                                                                                                                                                     S = S + 1
                                                                                                                                                                                                                                                                                                                                                                                                                                            Exit Do
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    Dim FileName$
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       End Function
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  foof ()
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    'Function:
                                                                                                                                                                                                                                                                                                      Seq (FP) +
                                                                                                                                                                                                                                                                                                                                                                                                                                                                          End If
                                                                                                                                                                                                                                                                                                                                                                                                                   Else
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            Seq (FP
If (TP
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 Next S
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                Ιţ
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Note carefully, these numbers are given for the sequence of the DNA strand 1.e. we assume that the probe is DNA, the target 'is RNA, and the sequence of the probe is given to the routine. For example, the parameter in (0,0) is for dAA/rTT. or 1G or GG AA Or TT
AA Or GT
AA Or CT
AA Or CT
CA Or TG
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CG CG CG or 11 or G1 or IC 'Initialize the DNA/RNA duplex parameters. 'Parameters found in Sugimoto et al., Blochemistry, 'v. 34, pp. 11,211-11,216 (1995). UNA_DUPLEXS(0, 2) = -16.1
UNA_DUPLEXS(0, 3) = -18.8
UNA_DUPLEXS(1, 0) = -19.3
UNA_DUPLEXS(1, 1) = -15.6
UNA_DUPLEXS(1, 2) = -25.5
UNA_DUPLEXS(1, 2) = -25.5
UNA_DUPLEXS(1, 2) = -26.3
UNA_DUPLEXS(1, 2) = -26.3
UNA_DUPLEXS(2, 1) = -28.4
UNA_DUPLEXS(2, 1) = -28.4
UNA_DUPLEXS(2, 1) = -28.4
UNA_DUPLEXS(2, 1) = -18.5
UNA_DUPLEXS(2, 1) = -18.5
UNA_DUPLEXS(3, 1) = UNA_DUPLEXS(1, 1)
UNA_DUPLEXS(3, 1) = UNA_DUPLEXS(1, 0)
UNA_DUPLEXS(3, 1) = UNA_DUPLEXS(1, 0)
UNA_DUPLEXS(3, 1) = UNA_DUPLEXS(1, 0)
UNA_DUPLEXS(3, 2) = UNA_DUPLEXS(1, 0)
UNA_DUPLEXS(3, 3) = UNA_DUPLEXS(1, 0)
UNA_DUPLEXS(3, 3) = UNA_DUPLEXS(1, 0)
UNA_DUPLEXS(3, 3) = UNA_DUPLEXS(1, 0) 2 == 888 = DNA_DuplexH(0, 2) = -7.7== 888 DNA_DuplexH(1,DNA_DuplexH(0, DNA_DuplexH(2, DNA DuplexH(1, DR_DuplexH(0, 0) = -11.5
DR_DuplexH(0, 1) = -7.8
DR_DuplexH(0, 3) = -9.3
DR_DuplexH(1, 0) = -10.4
DR_DuplexH(1, 0) = -10.4
DR_DuplexH(1, 1) = -12.8
DR_DuplexH(1, 2) = -16.3
DR_DuplexH(1, 3) = -9.1
DR_DuplexH(1, 3) = -9.1 = -7.4 = -6.7 = -10.1 -11:1 DNA_InitGCS = -5.9 DNA_Duplexs(0, 0)
DNA_Duplexs(0, 1)
DNA_Duplexs(0, 2) DNA_InitATS = -9 DNA_SelfS = -1.4 DNA_EndTAH = 0.4 DNA DuplexH(0, DuplexH (0,

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1. This routine reads data files originally designed to be read by MFOLD; the files must be edited to place a # sign on lines that do not contain data, and to replace all the "." entiles by 0.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             presented.

3. The FirstCol argument is used only by Loop and TetraLoop types.

4. In all these files, the order of bases is ACGU->0,1,2,3
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   2. There are several types of parameter files. Type "stack" gives the parameters for stacking one base pair over another, and thus has 256 entries. Type "dangle" gives the parameters for dangling a base over a pair, and thus has 64 parameters.
                                                                                                                                                                                                                                   FileName = App.Path & "\tstackh.dat.pw"
ReadZuker FileName, "Stack", RNAMP_TStackG, foo
FileName = App.Path & "\tstackh.dh.pw"
ReadZuker FileName, "Stack", RNAMP_TStackH, foo
CalcsFromGH 4, RNAMP_TStackH, 37#, RNAMP_TStackS
                                                                                                                                                                                                                                                                                                                                                                                                         FileName = App.Path & "\dangle.dat.pw"
ReadZuker FileName, "Dangle", RNAMP DangleG, foo
FileName = App.Path & "\dangle.dh.pw"
ReadZuker FileName, "Dangle", RNAMP DangleH, foo
CalcSFromGH 4, RNAMP DangleS, RNAMP DangleS
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  Private Sub ReadZuker(FileName$, ZType$, Param#(), FirstCol&())
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               The other types are particular to the parameters being
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      Read thermodynamic parameters for structure calculations.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   ReadZuker FileName, "Tetraloop", RNAHP_TLoopG, ZTetraloops
FileName = App.Path & "\tloop.dh.pw"
ReadZuker FileName, "Tetraloop", RNAHP_TLoopH, ZTetraloops
CalcSFromGH 1, RNAHP_TLoopG, RNAHP_TLoopH, 37#, RNAHP_TLoopS
                                                                                          ReadLuker Filename, "Stack", RNAHP StackG, foo
FileName = App.Path & "\stack.dh.pw"
ReadZuker FileName, "Stack", RNAHP_StackH, foo
CalcsFromGH 4, RNAHP_StackG, RNAHP_StackKH, 37#, RNAHP_StackS
CalcsFromGH 1, DNAMP_TLoopG, DNAMP_TLoopH, 37#, DNAMP_TLoopS
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             ReadZuker FileName, "Loop", RNAHP_LoopG, ZLoopLengths FileName = App.Rath ("NIOPO-Lh.pw" REAdZuker FileName, "Loop, RNAHP_LoopH, ZLoopLengths ReadZuker FileName, "LoopG, RNAHP_LoopH, 37%, RNAHP_LoopS
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           21ype: The type of parameter array (see below). Param: The parameter array to be filled in. FirstCol: The values from the first column.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           FileName - App.Path & "\tloop.dat.pw"
                                                                  FileName = App.Path & "\stack.dat.pw"
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     FileName = App.Path & "\loop.dat.pw"
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      Filename: The data file.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              *Arguments
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       Funct ion
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FileName - App.Path & "Ndangle.dhd.by"
Fradzuker FileName, "Dangle", DNAHP_DangleH, foo
CalcSFromGH 4, DNAHP_DangleG, DNAHP_DangleH, 37*, DNAHP_DangleS
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        Readzuker FileName, "Stack", DNAHP_TStackH, foo
CalcsFromGH 4, DNAHP_TStackG, DNAHP_TStackH, 37%, DNAHP_TStackS
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     foo
374, DNAHP_StackS
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ReadZuker FileName, "Tetraloop", DNAMP_TLoopG, ZTetraloops
FileName = App.Path & "\tloop.dhd.pw"

ReadZuker FileName, "Tetraloop", DNAMP_TLoopH, ZTetraloops
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              FileName = App.Path 6 "\loop.datd.pw"

ReadZuker FileName, "Loop", DNAHP LoopG, ZLoopLengths
FileName = App.Path 6 "\loop.dhd.pw"

ReadZuker FileName, "Loop", DNAHP_LoopH, ZLoopLengths
CalcSFromGH 2, DNAHP_LoopG, DNAHP_LoopH, 37$, DNAHP_LoopS
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              FileName = App.Path & "\tstackh.datd.pw"
Readzuker FileName, "Stack", DNAHP TStackG, foo
FileName = App.Path & "\tstackh.dhd.pw"
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  *Load the Zuker hairpin parameters.
Filename = App.Path & "\stack datd.pw"
ReadZuker Filename, "Stack", DNAHP_StackG, foo
Filename = App.Path & "\stack dhd.pw"
ReadZuker Filename, "Stack", DNAHP_StackH, foo
CalcSFromGH 4, DNAHP_StackG, DNAHP_StackH, 374,
                                                                                                                                                                                                                                                                                                                    8888888
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                FileName = App.Path & "\dangle.datd.pw"
                                                                                                                                                                                                                                                                                                                       DR_Duplexs(0, 0) = -36.4
DR_Duplexs(0, 1) = -21.6
DR_Duplexs(0, 2) = -21.9
DR_Duplexs(0, 3) = -23.9
DR_Duplexs(1, 0) = -28.4
DR_Duplexs(1, 0) = -28.4
DR_Duplexs(1, 2) = -47.1
DR_Duplexs(1, 3) = -23.5
DR_Duplexs(2, 0) = -22.9
DR_Duplexs(2, 1) = -17.1
DR_Duplexs(2, 2) = -73.2
DR_Duplexs(2, 3) = -17.1
DR_Duplexs(3, 3) = -12.3
DR_Duplexs(3, 1) = -13.3
DR_Duplexs(3, 1) = -13.5
DR_Duplexs(3, 1) = -13.5
                                                                                 DR_DuplexH(2, 1)
DR_DuplexH(2, 2)
DR_DuplexH(2, 3)
DR_DuplexH(3, 0)
DR_DuplexH(3, 1)
DR_DuplexH(3, 2)
DR_DuplexH(3, 3)
DR_DuplexH(3, 3)
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                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               DR_Inits = -3.9
                                                                                                                                                                                                                                                                 DR_InitH = 1.9
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Attorney Docket No. 10971464-1

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FirstCol(L) = 0
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                                                                                                                                                                                                                                                                                                                                                                                                                                                                     Case "Loop"
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Next A
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              'By row, A varies.
'Index order used is param(0,A,X,B) for 3', and param(1,A,B,Y) for the 5'
                                                                                                                                                                                                                                                                                                                                                             Dim At. Bt. Xt, Xt, Lt. Ct. Tt 'loop indices
Dim NumSeq*(0 To 3) 'numeric representation of tetraloop sequences
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      For B = 0 To 3
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For Y 
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             These entries represent stacking of one base pair over another:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        These entries represent dangles of 3' ends, then the 5' ends:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     NextWord LineStr, LineLength, WordStr, WordLength
Param(0, A, X, B) = CDbl(WordStr)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  For x = 0 To 3
NextLine FileStr, FileLength, LineStr, LineLength, **
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    NextLine Filestr, FileLength, LineStr, LineLength, "#"

    5'-A -3'
    3'-BY-5' is a 5' dangle.
    in each row, X or X varies fast, and B varies slowly.

                                                                                                        ·length of parameter file
·length of one line
·length of one word
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          'In each row, Y varies fast, B varies slow.
'By row, X varies fast, and A varies slowly.
'Index order used is param(A, X, B, Y).
                                                                                                                                                                                                                                       text of read-in file
                                                                                                                                                                                                                                                                                         text of one line text of one word
                                                                    parameter file
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      Filestr = Input(FileLength, #ZFile)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           Open FileName For Binary As #ZFile
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            'Choose how to parse the file.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         FileLength - FileLen (FileName)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    3'-B -5' is a 3' dangle
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              ·Open the file, read it in.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            For X = 0 To 3
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              Next Y
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              Select Case 2Type
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                ZFile - FreeFile
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         dangles.
For A = 0 To 3
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     Next X
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           Next B
                                                                                 Dim ZFile6
Dim FileLength6
                                                                                                                                                                       Dim LineLength&
                                                                                                                                                                                                            Dim WordLength&
Dim FileStr$
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              Case "Dangle"
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    Case "Stack"
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   3'-BY-5'
                                                                                                                                                                                                                                                                                                  Dim LineStr$
Dim WordStr$
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    Next X
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      Next A
```

'By row, looplength L varies.
'Index order used is param(looptype, L)
'FirstCol is filled from the first column, representing loop lengths.
For L = 0 To NumzukerLoops = 1 These entries represent internal=0, bulge=1, and hairpin=2 loops. calculate the association enthalpy for a given RNA sequence with its perfect W-C DNA complement. For Y = 0 To 3 NextWord LineStr, LineLength, WordStr, WordLength Param(1, A, B, Y) = CDb1(WordStr) These represent especially stable tetraloops.

By row, sequence of the tetraloop varies.

Firstcol is filled with a quaternary index of the sequence.

For L = 0 To NumTetraLoops - 1 NextLine Filestr, FileLength, LineStr, LineLength, "#" NextWord LineStr, LineLength, WordStr, WordLength NextLine Filestr, FileLength, Linestr, LineLength, "NextWord Linestr, LineLength, Wordstr, WordLength FirstCol(L) = CLng (WordStl)
For T = 0 To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C T No To C For A = 0 To 3

NextLine FileStr, FileLength, LineStr, LineLength, ***
For B = 0 To 3 Nextword LineStr, LineLength, WordStr, WordLength Param(L) = CDbl(WordStr) FirstCol(L) = FirstCol(L) * 4 + NumSeq(C) 1. Standard conditions (IM NA+) is assumed. Enthalpy of association, in kcal/mole. Function DR_CalcDeltaH#(ByVal seq\$) DNA_Str2Num WordStr, NumSeq seq: The RNA sequence.

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Attorney Docket No. 10971464-1

Attorney Docket No. 10971464-1

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Attorney Docket No. 10971464-1
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     'Sum the nearest neighbor values along the strand
For B = 1 To Length - 1
DNA_CalcDeltaS = DNA_CalcDeltaS + DNA_DuplexS(NumSeq(B - 1), NumSeq(B))
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        'Initialize with self-symmetry correction.
If seq = DNA_RevComp(Seq) Then DNA_CalcDeltas = DNA_CalcDeltas + DNA_Selfs
                                              'Sum the nearest neighbor values along the strand. For B = 1 To length - 1 DA_CalcDeltaH + DNA_DuplexH(NumSeq(B - 1), NumSeq(B))
If Numseq(Length - 1) = 0 Then DNA_calcDeltaH = DNA_calcDeltaH + DNA_EndTAH

    Parameters are derived from Santalucia et al., Biochemistry,
    v. 35, pp. 3555-3562 (1996).
    Standard conditions (1M NA+) is assumed.

                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         'length of the sequence
'base index for traversing the sequence
'numerical representation of the sequence
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            .
М.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              29-Jul-1997: From PkW's routine of the same name.
                                                                                                                                                                                                                                                                                                                                                                                                                                                     calculate the association entropy for a sequence with its perfect W-C complement.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             'add initiation term
If Instr(Seq, "c") Or Instr(Seq, "g") Then
DNA_CalcDeltaS = DNA_CalcDeltaS + DNA_InitGCS
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                DNA_CalcDeltaS = DNA_CalcDeltaS + DNA_InitATS
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               Entropy of association, in cal/mole/deg K.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       72
                                                                                                                                                                                                                         DNA_CalcDeltaH = DNA_CalcDeltaH * 1000$
                                                                                                                                                                                                                                                                                                                                                                                      Function DNA_CalcDeltaS#(ByVal Seq$)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 "Create the numeric representation.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       Copyright Hewlett-Packard Company, 1998
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          Length = Len(Seq)
ReDim NumSeq(Length = 1)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    DNA_str2Num Seq, NumSeq
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        Seq: The sequence
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          'Lower case, please.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                Sed = ICase (Sed)
                                                                                                                                                                                                      Convert to cal.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                Dim NumSeq*()
                                                                                                                                                                                                                                                                                      End Function
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    Dim Length
                                                                                                                                                                                                                                                                                                                                                                                                                                                  Function
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    *Returns:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              'H1story
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        Notes
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                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                For B = 1 To Length - 1
DR CalcDeltaH = DR_CalcDeltaH + DR_DuplexH(NumSeq(B - 1), NumSeq(B))
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                'Initialize with AT end correction. If NumSeq(0) = 3 Then DNA_CalcDeltaH + DNA_EndTAH If NumSeq(0) = 3 Then DNA_CalcDeltaH = DNA_CalcDeltaH = -
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  base index for traversing the sequence inumerical representation of the sequence
                                                                                                           ·length of the sequence
·base index for traversing the sequence
·numerical representation of the sequence
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               P.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          Calculate the association enthalpy for a DNA sequence with its perfect W-C complement.
                        29-Jul-1997: From PkW's routine of the same name. PW
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               29-Jul-1997: From PkW's routine of the same name.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               'Sum the nearest neighbor values along the strand.

    Standard conditions (1M NA+) is assumed.

                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               'length of the sequence
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      Enthalpy of association, in kcal/mole.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         'Convert to cal.
DR_calcDeltaH = DR_calcDeltaH * 1000#
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             Function DNA_CalcDeltaH#(ByVal Seq$)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       *Create the numeric representation.
                                                                                                                                                                                                                                                                                                      *Create the numeric representation.
                                                                                                                                                                                                                                                                                                                                                                                                                                  'Initialize with initiation deltaH
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       © Copyright Hewlett-Packard Company, 1998
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                Length = Len(Seq)
ReDim NumSeq(0 To Length - 1)
                                                                                                                                                                                                                                                                                                                                                  ReDim NumSeq(0 To Length - 1)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       DNA StrZNum Seq, NumSeq
                                                                                                                                                                                                                                                                                                                                                                                                                                                                   DR_CalcDeltaH = DR_InitH
                                                                                                                                                                                                                                                                                                                                                                                      DNA_Str2Num Seq, NumSeq
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             Seq: The sequence.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               Lower case, please.
                                                                                                                                                                                                                            Lower case, please
                                                                                                                                                                                                                                                                                                                                       Length = Len(Seq)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        Seq = LCase (Seq)
                                                                                                                                                                                                                                                       Sed = ICase(Sed)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        Dim NumSeq*()
                                                                                                                                                                       Dim NumSeq*()
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      End Function
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    Dim Length
                                                                                                                   Dim Length&
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       ·Function
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      Returns
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            "History
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          Notes
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       Next B
```

```
For SS = TClamp.FiveP + 1 To Len(Seq(S)) - TClamp.ThreeP - TClamp.Length + 1
SubSeq = Mid(Seq(S), SS, TClamp.Length)
ThisIM = DNA_CalcTM(SubSeq, TClamp.Conc) + 273.15
If ThisIM > BestIM Then BestIM = InisIM
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     Attorney Docket No. 10971 464-1
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                Tolamp: An instance of the parameter class for Clamp calculations.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              1. Concentration is used as is, i.e. assuming the complement is present at much lower concentration.
                                                                                                                                                                                                                                                                                                                                                                                                                                                          Public Sub DNA_calcclamp(Seq$(), Tclamp As cclampPars, Clamp#())
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          'number of sequences we are working with
                                     'number of sequences we are working with 'index
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              'Determine the number of probes we are calculating Clamp for.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   Calculate the melting temperature of the Clamp of a probe
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   For S = 0 To NumSeqs - 1
If S - Progress.StopAt = 0 Then Progress.CheckProgress S
                                                                                                                                                                                             'Calculate melting points
For S = 0 To Numbegs = 1
If S - Progress.StopAt = 0 Then Progress.CheckProgress S
TM(S) = DNA_CalcTM(Seq(S), tmp.Conc)
                                                                                                            'Determine the number of probes we are calculating TM for.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     Melting temperature of tightest clamp, in deg.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   current most stable clamp
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                E: Debug.Print "Error in DNA_CalcClamp"
Err.Raise Err.Number, , Err.Description
End Sub
                                                                                                                                                                                                                                                                                                                                                       E: Debug.Print "Error in DNA_CalcaliTM"
Err.Raise Err.Number, , Err.Description
End Sub
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            7
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               epuendesque,
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      to its perfect W-C complement
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               Copyright Hewlett-Packard Company, 1998
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        'indices
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               'Calculate melting points.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          NumSeqs = UBound (Seq) + 1
                                                                                                                                             NumSeqs = UBound(Seq) + 1
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    Seq: The sequences
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     DIM BestTM#, ThisTM#
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     Clamp(S) = BestTM
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 On Error GoTo E
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                BestIM = 0
On Error GoTo
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    Dim NumSeqs£
                                                Dim NumSeqs&
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           Dim Se, SS6
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 Dim SubSeq$
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 Next SS
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      Funct 1on
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      *Returns
                                                                                                                                                                                                                                                                                                                 Exit Sub
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               Next S
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      *Notes
                                                                           Dim S&
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      Attorney Docket No. 10971464-1
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    DR_CalcDeltas = DR_CalcDeltas + DR_DuplexS(NumSeq(B = 1), NumSeq(B))
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 Calculate the melting temperature of an array of oligos with their

    Concentration is used as is, i.e. assuming the complement
is present at much lower concentration.

                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    TMP: An instance of the parameter class for TM calculations.
                                                                                                                                                                                                     calculate the association entropy for a given RNA sequence with its perfect W-C DNA complement.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     length of the sequence
thase index for traversing the sequence
thumerical representation of the sequence
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                ¥.
                                                                                                                                                                                                                                                                                                                                                                                                                                Z.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             Public Sub DNA_CalcallTM(Seq$(), tmp As cTMPars, TM#())
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  29-Jul-1997: From PkW's routine of the same name.
                                                                                                                                                                                                                                                                                                                                                                                                            'History 29-Jul-1997; From PkW's routine of the same name.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           'Sum the nearest neighbor values along the strand.
                                                                                                                                                                                                                                                                                                                                                                              . Standard conditions (1M NA+) is assumed
                                                                                                                                                                                                                                                                                                                              Entropy of association, in cal/mole/deg K.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              5
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           Melting temperature, in deg. C.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   *Create the numeric representation.
                                                                                                                                   Function DR_CalcDeltaS# (ByVal Seq$)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              Copyright Hewlett-Packard Company, 1998
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                ReDim NumSeq(0 To Length - 1)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     Begin with initiation term.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       perfect W-C complements.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                DR_calcDeltas = DR_Inits
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          DNA Str2Num Seg, NumSeg
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       Seq: The sequences
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     For B = 1 To Length -
                                                                                                                                                                                                                                                                                     Seq: The sequence
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            Lower case, please.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            Length = Len(Seq)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                Seq = LCase (Seq)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            Dim NumSeq*()
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          End Function
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            Dim Lengthé
Dim Bé
                    End Function
                                                                                                                                                                                                                                                       Arguments
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    'History
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          Function
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          Returns
                                                                                                                                                                                        Function
                                                                                                                                                                                                                                                                                                               *Returns:
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          Notes
                                                                                                                                                                                                                                                                                                                                                               Notes
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OGFOWS LOCATOR

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BestTM = 0
For SS = TClamp.FiveP + 1 To Len(Seq(S)) = TClamp.ThreeP = TClamp.Length + 1
Subseq = Mid(Seq, SS, TClamp.Length)
ThisTM = DR CalcTM(Subseq, TClamp.Conc) + 273.15
If ThisTM > BestTM Then BestTM = ThisTM
                                                                                                                                                                                            TClamp: An instance of the parameter class for Clamp calculations
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         dGHP: An instance of the parameter class for dGH calculations dGH: The hairpin dGs.

    Concentration is used as is, i.e. assuming the complement
is present at much lower concentration.

Public Sub DR_Calcclamp(Seq$(), TClamp As cclampPars, Clamp*())
                                                                                                                                                                                                                                                                                                                                                                                                                                               'number of sequences we are working with
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        'Recalculate all G parameter matrices at current temperature.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             'Determine the number of probes we are calculating Clamp for.
                                                                                   Calculate the melting temperature of the Clamp of a probe
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  If S - Progress.StopAt = 0 Then Progress.CheckProgress S
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          Public Sub DNA_CalcdGH(Seq$(), dGHP As cDGHPars, dGH#())
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    'numeric representation of the sequence
'index
                                                                                                                                                                                                                                                      Melting temperature of tightest clamp, in deg. C.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           Calculate the hairpin dGs of an array of oligos.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              Dim Subseq$ 'subsequence
Dim BestTM*, ThisTM* 'current most stable clamp
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            Err.Raise Err.Number, , Err.Description
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     E: Debug.Print "Error in DR_calcclamp"
                                                                                                                   to its perfect RNA W-C complement.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       ·indices
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      *Calculate melting points.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               Numseqs = UBound(Seq) + 1
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              Seq: The sequences.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   For S = 0 To NumSeqs -
                                                                                                                                                                             Seq: The sequences
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      Clamp(S) = BestTM
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            On Error GoTo E
                                                                                                                                                                                                                                                                                                                                                                                                         On Error GoTo E
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                Dim NumSeq*()
                                                                                                                                                                                                                                                                                                                                                                                                                                                             Dim NumSeqs6
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  Next SS
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          Dim St, SSt
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               Funct 1on
                                                                                                                                                         'Arguments
                                                                                                                                                                                                                                                                                           Notes
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CalcGrowns 4, DNAMP_StackH, DNAMP_StackS, dGHP.T, DNAMP_StackG calcGrowns 4, DNAMP_TStackD CalcGrowns 4, DNAMP_DTSTACKH, DNAMP_DTSTACKB, dGHP.T, DNAMP_DTSTACKG CALCGFTOWNS 4, DNAMP_DANGIAH, DNAMP_DANGIAS, dGHP.T, DNAMP_LOOPG CALCGFTOWNS 2, DNAMP_LOOPH, DNAMP_LOOPS, dGHP.T, DNAMP_LOOPG CALCGFTOWNS 1, DNAMP_TLOOPG CALCGFTOWNS 1, DNAMP_TLOOPG
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   dGDP: An instance of the parameter class for dGD calculations.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     seq: The sequences.
dGDP: An instance of the parameter class for dGD calculations.
dGD: The hairpin dGs.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             If S - Progress StopAt = 0 Then Progress CheckProgress S dGD(s) = (DNA_CalcoaltaH(Seq(s)) - (dGDP.T + 273.15) * DNA_CalcoeltaS(Seq(s)) / 1000#
                                                                                                                                                                                                                                               If S - Progress.StopAt = 0 Then Progress.CheckProgress S ReDim Numseq(0 To Len(Seq(S)) - 1)
DNA_Str2Num Seq(S), NumSeq
dGH(S) = DNA_BestHalrpin(NumSeq)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                'numeric representation of the sequence
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          Public sub DNA_CalcdGD(Seq$(), dGDP As cdGDPars, dGD*())
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               'numeric representation of the sequence 'index
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            Public Sub DR_CalcdGD(Seq$(), dGDP As cdGDPars, dGD#())
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    Calculate the duplex dGs of an array of oligos.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   Calculate the duplex dGs of an array of oligos
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       E: Debug.Print "Error in DNA_CalcGD"
Err.Raise Err.Number, , Err.Description
                                                                                                                                                                                                                                                                                                                                                                                                                                                                        Err. Raise Err. Number, , Err. Description
                                                                                                                                                                                                                                                                                                                                                                                                                                      E: Debug.Print "Error in DNA_CalcGH"
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                For S = 0 To UBound (Seq)
                                                                                                                                                                                                                               For S = 0 To UBound(Seq)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         Seq: The sequences.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              On Error GoTo E
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                On Error GoTo E
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    'Calculate dGs.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  calculate dGs
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        Dim NumSeq ()
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          Dim NumSeq*()
                                                                                                                                                                                                         Calculate dGs
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             *Arguments
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            Arguments
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 Funct 1on
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   *Function
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              End Sub
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Next
      If s = Progress.StopAt = 0 Then Progress.CheckProgress S
dGD(s) = (DR_CalcDeltaH(Seq(s)) - (dGDP.T + 273.15) * DR_CalcDeltaS(Seq(s)))
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  'Recalculate all G parameter matrices at current temperature. CalcGFrom45 4, RNAHP_StackH, RNAHP_StackS, GGHE.T, RNAHP_StackG CalcGFrom45 4, RNAHP_TStackH, RNAHP_TStackS, GGHE.T, RNAHP_TSTackG CalcGFrom45 4, RNAHP_DangleH, RNAHP_DangleS, GGHP.T, RNAHP_DangleG CalcGFrom45 2, RNAHP_LoopH, RNAHP_LoopS, GGHP.T, RNAHP_LoopG CalcGFrom45 1, RNAHP_TLoopG CalcGFrom45 1, RNAHP_TLoopG
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 dGHP: An instance of the parameter class for dGH calculations.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                              Seq: The sequences.
dGMP: An instance of the parameter class for dGM calculations.
dGM: The hairpin dGs.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               If s - Progress.StopAt = 0 Then Progress.CheckProgress S
dGM(s) = firmWaln.Mfoldx.mfold(Seq(S), Val(dGNE.T), True)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               Public Sub RNA_CalcdGH(Seq$(), dGHP As cDGHPars, dGH*())
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  'numeric representation of the sequence
                                                                                                                                                                                                                                                                                                               Public sub DNA_calcdGM(Seq$(), dGMP As cdGMPars, dGM#())
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         Calculate the hairpin dGs of an array of oligos
                                                                                                                                                                                                                                                                                                                                                                                                            Calculate the MFold dGs of an array of oligos.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          E: Debug.Print "Error in DNA_CalcdGM."
Err.Raise Err.Number, , Err.Description
                                                                                                                                                                       E: Debug.Print "Error in DNA_CalcGD"
Err.Raise Err.Number, , Err.Description
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     For S = 0 To UBound (Seq)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          · index
For S = 0 To UBound (Seq)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           · index
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   On Error GoTo E
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  *Calculate dGs
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   On Error GoTo E
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      'Calculate dGs.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 Dim NumSeq*()
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       Arguments
                                                                                                                                                                                                                                                                                                                                                                                                                                                     'Arguments
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             Function
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           Exit Sub
                                                                                                                                                                                                                                                                                                                                                                                          Funct 1on
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              Dim SE
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calculate the melting temperature of an array of DNA probes with their
                                                                                                                                                                                                                                                                                                                                                                                                                                           Seq: The sequences.
dGMP: An instance of the parameter class for dGM calculations.
dGM: The MFold dGs.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      1. Concentration is used as is, i.e. assuming the complement
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            Seq: The sequences.
TMP: An instance of the parameter class for TM calculations.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          'number of sequences we are working with
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                For S = 0 To UBound(Seq)
If S - Progress.StopAt = 0 Then Progress.CheckProgress S
dGM(s) = firmMain.Mfoldx.mfold(Seq(S), Val(dGMP.T), False)
For S = 0 To Ubound(Seq)

If S - Progress.StopAt = 0 Then Progress.CheckProgress S ReDIm NumSeq(0 To Len(Seq(S)) - 1)

UNL StrZhum Seq(S), NumSeq

GGH[S) = RNA_BestHaltpln(NumSeq)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      'Determine the number of probes we are calculating TM for. Numseqs = UBound(Seq) - LBound(Seq) + 1
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          ž
                                                                                                                                                                                                                                                                                             Public sub RNA_CalcdGM(Seq$(), dGMP As cdGMPars, dGM#())
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 Public Sub DR_CalcallTM(Seq$(), tmp As cTMPars, TM#())
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          29-Jul-1997: From PkW's routine of the same name.
                                                                                                                                                                                                                                                                                                                                                                                Calculate the MFold dGs of an array of oligos
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     is present at much lower concentration.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          E: Debug.Print "Error in RNA_CalcGW" .
Err.Raise Err.Number, , Err.Description
End Sub
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      perfect W-C RNA target complements.
                                                                                                                                                                                                                 Err. Raise Err. Number, , Err. Description
                                                                                                                                                                                     E: Debug.Print "Error in RNA_CalcdGH"
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        Melting temperature, in deg. C.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         'index
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          · 1ndex
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         On Error GoTo E
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       On Error GoTo E
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               'Calculate dGs.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        Dim NumSeqs6
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        'Arguments
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      Function
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       Returns
                                                                                                                                                                                                                                                                                                                                                                 Funct ion
                                                                                                                                                                         Exit Sub
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          Notes
                                                                                                                                                                                                                                                      End Sub
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            Dim S&
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Attorney Docket No. 10971464-1

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Attorney Docket No. 10971 464-1
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               'Mark elements, stepping down from the middle first. This ensures that all 'elements get marked for even lengths, spacing-i. Steppir = -1
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       'Record the ends, step to the middle (to the right of middle for even
                                                                                                                                                              Do While ((P - EndDown) = (Pos(P) - Pos(EndDown)))
EndDown = EndDown - 1
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           P = P + StepDir * M * RP.Spacing
If (P < EndDown Or P > EndUp) Then Exit For
                                                                                                                                                                                                                                                                                                                                         Do while ((Endup - P) = (Pos(Endup) - Pos(P)))
                                                                                                                                                                                                                                                                                                                                                                                                 If EndUp = UBound(Pos) + 1 Then Exit Do
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              'Convert marked elements back to run length.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        'Find next run.
Do While Run(P) = 0 And P < UBound(Run)
P = P + 1
current step direction
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        'Pick out requested elements from each run
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      If Run (P) > 0 Then Run (P) = 0
If Run (P) < 0 Then Run (P) = -Run(P)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            P = F1x((EndDown + EndUp + 1) / 2)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      8
                                                                                                                                                                                                                               If EndDown = -1 Then Exit Do
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           'Remove runs that are too short.
For P = 0 To UBound(Run)
If Run(P) < RP.Min Then Run(P)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      Copyright Hewlett-Packard Company, 1998
                                                                                                                                                                                                                                                                                                                                                                                                                                                                 EndUp = EndUp - 1
Run(P) = EndUp - EndDown + 1
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            StepDir = StepDir * -1
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  + Run(P) - 1
                                                                                                                                                                                                                                                                                          EndDown = EndDown + 1
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       Run (P) = -Run (P)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 Move over this run.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 For P = 0 To UBound (Run)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               For M = 1 To RP.Num
                                                                                                                                                                                                                                                                                                                                                                               Endup - Endup +
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   Do While P < UBound (Run)
                                                                                  *Loop over positions.
For P = 0 To UBound(Pos)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               P = EndUp + 1
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       EndDown = P
                                                                                                                                          EndDown = P
  Dim StepDire
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               Next M
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   lengths)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               Next P
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      Next P
                                Dim Me
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            Attorney Docket No. 10971464-1
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       Begin by looping over all positions, extending down over all consecutive Begin by looping over all consecutive entries. Then mark in the active entries, then up over all consecutive entries. Then mark in the active too short. Finally, for each trun, mark the requested number of members of the run, at the requested spacing, as being members. If the run is shorter than can accomdate the requested number of members, fill in as many as possible, while still preserving the requested spacing.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    DNA_CalcTM = DNA_CalcDeltaH(Seq) / (DNA_CalcDeltaS(Seq) + RGas * Log(Conc)) -
                                                                                                                                                                                                                                                                                                                                                                                                                                          DR_CalcTM = DR_CalcDeltaH(Seq) / (DR_CalcDeltaS(Seq) + RGas * Log(Conc))
                                                                                                                                                                                                                                                                                                                                                                                     Calculate the TM of one DNA/RNA duplex. Sequence given is DNA
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               1. Run must be allocated and sized correctly by the caller.
                                                                   If S - Progress.StopAt = 0 Then Progress.CheckProgress 5
IM(S) = DR_CalcIM(Seq(S), tmp.Conc)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            'A quicksort routine for doubles, based on NR code. Private Declare Function vb5sort2 Lib "vb5nrdll.dll"
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       (Byval NE, ByRef Vector#, ByRef Index4) As Long
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        end of the run, counting up
end of the run, counting down
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         Public Sub CalcRun(Post(), RP As CRunPars, Runt())
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 Calculate the runs in a set of positions.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     Calculate the TM of one DNA/DNA duplex
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     Public Function DNA_CalcTM# (Seq$, Conc#)
                                                                                                                                                                                      E: Debug.Print "Error in DR_CalcAllIM" Err.Raise Err.Number, , Err.Description
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     8
                                                                                                                                                                                                                                                                                                        Public Function DR_CalcTM# (Seq$, Conc#)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        Copyright Hewlett-Packard Company, 1998
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             Attribute VB_Name = "Utilities"
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         RP: The run parameters. Run: The returned run.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   ·1ndex
              'Calculate melting points
For S = 0 To NumSeqs - 1
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         Pos: The positions.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             Option Explicit .
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             End Function
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      27<u>3</u>.15
End Function
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               Funct 1on
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   'Function
                                                                                                                                                                                                                                                                                                                                                                         Funct 1on
```

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Public Function StrField$(Str$, lineLen$, ByVal FieldLen$, Optional Extra$)
Returns a leading field of a string, and shortens the string
'by the fleatdength + extra.
'If the line is too short, as much of the requested field as possible
'will be returned.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       Public sub NextLine(Filestrs, FileLens, LineStrs, lineLens, CommentChars)
'Return the next line that doesn't begin with CommentChar
lineLen = 0
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           'strips leading spaces, copies leading word, reduces linelength Do While ((Wid(Str, l, l) = " *) And (StrLen > 0)) StrLen * StrLen * StrLen * 1
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     FileLen = FileLen - lineLen - 2
FileStr = Right (FileStr, FileLen)
If (Mids (LineStr, 1, 1) = CommentChar) Then lineLen = 0
                                                                                                                                                                                                                                                                                                                                                                                                                                                                             Public Sub NextWord (Strs, StrLens, Words, WordLens)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          'Quicksorts the array X into ascending order,
'simultaneously sorting I to provide a sort index.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                Public Sub QuickSort (X#(), Indexs(), LBs, UBs)
                                                                                                                                                                                If FleidLen > lineLen Then FleidLen = lineLen
SrrField = Trim(Left(Str, FleidLen))
lineLen = lineLen - FleidLen
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 lineLen = Instr(FileStr, vbCrLf) - 1
LineStr = Left(FileStr, lineLen)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       Do While (lineLen = 0 And FileLen > 0)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             82
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        foo = vb5sort2(N, X(LB), Index(LB))
                                                                                                                                                                                                                                                                                                                               If lineLen < 0 Then lineLen = 0
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             Copyright Hewlett-Packard Company, 1998
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  Word = Mid(Str, 1, WordLen)
StrLen = StrLen - WordLen
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         WordLen = InStr(Str, " ")
                                                                                                                                                                                                                                                                          If Not IsMissing(Extra) Then lineLen = LineLen = Extra
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       If Instr(Str, " ") <> 0 Then
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               Str - Right (Str, StrLen)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              Str - Right (Str, StrLen)
                                                                                                                                                                                                                                                                                                                                                                                         Str = Right (Str, lineLen)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           If WordLen <> 0 Then
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   Wordlen = Strlen
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       Wordlen = 0
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 Dim N£, foot
N = UB - LB + 1
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       StrLen = 0
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             Word = "
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             Str = **
                                                                                                                                                                                                                                                                                                                                                                                                                         End Function
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    End If
End Sub
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         End Sub
                                                                                                                                                                                                                                                                                                                                                               End If
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                End If
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            Else
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           Loop
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            Else
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             Attorney Docket No. 10971464-1
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      Public sub UnpackSequence(ByRef Seqstr As String, ByRef NumStr As String) 'Seqstr is assumed to hold a packed sequence.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           Do While Len(SeqStr) - Bases > 60
NumStr = NumStr & Format(Format(Bases, "#####"), "@@@@@") & vbCrLf
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  A side effect of this routine is to position the recordset at the
                                                                                                                                                                                               Check whether it is possible to access the records of recordset connected to the sequence, probeset, or probes table. No current record need exist for this function to return True.
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         Numstr = Numstr & Format (Bases, "#####"), "@@@@@")
For I = Bases To Len(Seqstr)
NewSeqstr = NewSeqstr & Mid(Seqstr, I, 1)
If (I Mod 10 = 0) Then NewSeqstr = NewSeqstr & " "
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         NewSeqStr = NewSeqStr & Mid(SeqStr, I, I)
If (I Mod 10 = 0) Then NewSeqStr = NewSeqStr & " "
                                                                                                                                                                                                                                                                                                                                                         If Iskull(RS) Then Exit Function
If (RS.EOF = True And RS.BOF = True) Then Exit Function
IsGoodRS = True
                                                                                                              Public Function IsGoodRS(RS As Recordset) As Boolean
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           Count the number of records in the recordset
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             Public Function NumRecords# (RS As Recordset)
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  8
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           Newseqstr = NewSeqstr + vbCrLf
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     C Copyright Hewlett-Packard Company, 1998
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  For I = Bases To Bases + 59
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     If Bases <> Len (SeqStr) Then
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                NumRecords - RS.RecordCount
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   Bases = Bases + 60
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    SeqStr = NewSeqStr
End Sub
                                                                                                                                                                                                                                                                                                                   On Error GoTo Err
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  first record.
                                                                                                                                                                                                                                                                                                                                                IsGoodRS - False
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      Dim NewSeqStr$
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         Dim Basess, It
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      RS.MoveF1rst
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          End Function
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        End Function
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         RS.MoveLast
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       NumStr = **
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                Next I
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 Bases = 1
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    Funct 1on
                                                          End Sub
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                End If
                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           Notes
  Next P
```



Public Function PackSequence\$(SeqStr\$)
Dim NewStr\$, Ch\$
Dim Is 1 To Len(SeqStr)
For I = 1 To Len(SeqStr)
Ch = Mid\$(SeqStr, I, 1)
If InStr("ACGTUacgtu", Ch) Then PackSequence = PackSequence 6 Ch
End Function Public Function BinarySearch# (Vector#(), Value#, Ut, Lt) Do While U <> L
BinarySearch = (U + L) / 2
BinarySearch = Value Then Exit Function
If Vector(BinarySearch) > Value Then
U = BinarySearch
Else
L = BinarySearch
End If Find the index of a value in a sorted array. Loop End Function

